## Tier II Summary (PMRA # 2181217, MRID 48574765) revised by the US EPA, March 14, 2014

*Note that the regulatory authorities have set different NOAELs/LOAELs based on different interpretation of body weight findings in males and chronic interstitial inflammation in females (see section IV – Evaluation, Summary and Conclusions)*

**Study Type: Chronic Toxicity/Oncogenicity Study - Rat**

IIA 5.5.1 and 5.5.2 – MRID 48574765, PMRA 2181217

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|---|---|
| **Report:** | Kaiser St. (2011)<br>MCW-2 TECH: 104-Weeks Combined Chronic Toxicity and Oncogenicity (Feeding) Study in the Rat.<br>Harlan Laboratories Ltd., Switzerland; unpublished report No. B80188, dated 19 July 2011. Sponsor reference No. R-23353<br>Dates of experimental work: 20 February 2008 to 25 February 2010 (allocation A); 20 February 2008 to 22 February 2009 (allocation B) |
| **Sponsor/Submitter:** | Makhteshim Chemical Works Ltd, Israel. |
| **Guidelines** | OECD No. 453, EEC Method B.32, and OPPTS 870.4300<br>Deviations: none |
| **GLP/ Compliance** | Yes. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. |

### Executive Summary

In an oral combined chronic toxicity/oncogenicity study in rats (MRID 48574765; PMRA 2181217), 4 groups of HANRcc Wist(SPF) rats (allocation A, oncogenicity animals), 50 males and 50 females each, were treated for 104 weeks with 0, 30, 200 or 1200 ppm (0/0, 1.4/1.7, 9.6/11.6, and 57.7/69.3 mg/kg bw/day in males/females) of fluensulfone (MCW-2 tech., 96.7%, Batch No. NLL6692-3) in their diets. A satellite group (allocation B, chronic toxicity animals) of 20 males and 20 females per group was similarly treated for 52 weeks at the same dose levels (0/0, 1.6/1.9, 11.0/13.1, 66.3/75.2 mg/kg bw/day in males/females).

Usual investigations were conducted on all animals (viability/mortality, clinical signs, food/water consumption, body weights, ophthalmoscopic, hematology, clinical chemistry, urinalysis, etc.). In addition, a functional observational battery (FOB) and locomotor activity assessments were performed on allocation B animals at week 52. In 5 allocation B animals per group, a part of the liver was used for enzyme activity determinations. After 52 and 104 weeks,

the right femur and the incisors of the maxilla of ten animals per sex and group were also taken for fluoride determination.

Treatment of Wistar rats with fluensulfone at dietary concentrations of 30, 200, 1200 ppm for up to 104 weeks had no effects on survival, clinical signs or ophthalmoscopic findings. Grip strength and the results of the functional observational battery were also not affected by treatment for 52 consecutive weeks. A slight decrease in overall locomotor activity was noted in high dose males. No increase in palpable nodules or masses was observed at any dose level at either the interim and terminal sacrifices, and no increase in any type of neoplastic finding was recorded at terminal sacrifice.

Food consumption in males treated with 1200 ppm was slightly lower during the first 4 weeks of treatment, but no further effects on food consumption were recorded thereafter. Water consumption was also increased in high dose males and females. Absolute mean body weight and body weight gain were decreased by the treatment in a dose-dependent manner in males treated at 200 and 1200 ppm; however, the changes at 200 ppm were marginal (5-7%), although statistically significant for some weeks. At 1200 ppm, body weight and body weight gain were decreased by 11% and 15%, respectively, at study termination. In high dose females, body weight was decreased during the last 11 weeks of the study (decreased by 6% at study termination) and body weight gain was reduced from week 2 onwards (decreased by 13%).

After 52 weeks a number of changes in hematology and biochemistry parameters were noted, mainly in animals treated with 1200 ppm. At 200 ppm and above, slightly decreased prothrombin time (5%) was noted in males. Dose-dependent decreases in neutrophil and white blood cell counts were observed in all female dose groups. Clinical chemistry findings in the low and mid dose groups included changes in ALAT, sodium, chloride, calcium, phosphorus, total protein and globulin, in males and/or females. Clinical chemistry and organ weight data revealed some effects on the liver, limited to the 1200 ppm group. In the absence of correlating histopathological findings these changes were considered to be non-adverse metabolic adaptations. Other organ weight changes noted in high dose groups included increased kidney weights in males and females, and increased adrenal weights in males. No effects on adrenals were noted at histopathology. During the urinalysis, increased ketones were observed in 1200 ppm males and females, and increased leukocytes in high dose males.

After 104 weeks a few changes in hematology parameters, mainly in red blood cell parameters, were noted in animals treated with 1200 ppm. Decreased hemoglobin concentration distribution width (HDW) was noted in females at 200 and 1200 ppm. Decreased mean corpuscular hemoglobin (MCH) was observed in high dose males, and decreases in mean corpuscular hemoglobin concentration (MCHC) were noted in all males at 78 weeks, as well as in 1200 ppm males and 200 and 1200 ppm females at 104 weeks. Neutrophil counts were also increased in high dose females, and eosinophil (EOS) levels were increased in high dose males.

At necropsy after 52 weeks, no gross lesions that could be attributed to treatment with fluensulfone were observed. Microscopically, hyperkeratosis of the esophagus of minimal severity was observed in animals of both sexes treated with 1200 ppm, but no abnormality was noted in lungs.

At necropsy after 104 weeks, an increased number of foci in the lungs was recorded in the treated animals, compared to controls, attaining statistical significance only in males at 1200 ppm. Enlarged livers were recorded in 4 females (versus one in the control group), as well as cysts in the livers of 8 females (versus 2 in the control group) and foci in the liver of 4 females (versus 0 in the control group) treated at 1200 ppm. In males, a slight increase in foci in the prostate was noted in mid and high dose groups (5 in each treatment group versus one in the control group). No correlative microscopic observations were noted in the prostate. Microscopically, an increased incidence of hyperkeratosis in the esophagus was recorded in animals of both sexes treated at 200 and 1200 ppm. Since the severity grade was minimal in most cases, the hyperkeratosis was considered to be of no toxicological significance, and possibly from a slight irritating effect of fluensulfone. The incidence of chronic interstitial inflammation in the lungs was increased in males and females treated at 1200 ppm and in females treated at 200 ppm but was only statistically significant for males at 1200 ppm. This lesion was characterized by focal/multifocal changes consisting of interstitial or intra-alveolar inflammatory cells associated with hypertrophied reactive type II pneumocytes, associated with the presence of foamy intra-alveolar macrophages, which was also increased in mean severity grade. There were no treatment-related hyperplastic changes or tumors recorded. At 104 weeks, slight increases were observed in chronic nephropathy, tubular basophilia and mononuclear foci in the kidneys and mononuclear infiltrates in the pharynx of females treated at 1200 ppm as compared to controls.

Investigations of liver enzymes from liver tissues demonstrated that the phase II enzymes uridine diphosphoglucuronosyl-transferase (UDPGT) and glutathione S-transferase (GST) were induced in 200 and 1200 ppm females and in 1200 ppm males, and epoxide hydrolase (mEH) was induced in all male and female dose groups. These enzymes were more significantly induced in females than males.

After 52 weeks of treatment, fluoride measurements revealed a marked dose-dependent increase, several fold above controls, of the fluoride content in the ashes from bones and teeth at 200 and 1200 ppm. Only very slight increases in fluoride content in bones (up to 28% above controls) were noted in 30 ppm animals. No increase of the fluoride content in the ashes from teeth was recorded in animals treated with 30 ppm.

After 104 weeks of treatment, the fluoride content in the ashes from bones and teeth of all treated animals was markedly increased. At 30 ppm, significant increases of 47-81% above controls were seen in bones and non-statistically significant increases of 20-66% were seen in teeth. Changes in fluoride levels were not considered adverse as there were no associated structural signs of dental fluorosis (discoloration of the teeth) or skeletal fluorosis (mobility problems, changes in external appearance of bones, changes in bone histopathology) at any dose.

The NOAEL was established at 200 ppm (9.6/11.6 mg/kg/day, M/F) based on decreased body weight in males, hematology and clinical chemistry findings in both sexes, histopathological changes in the lungs of males, and histopathological changes in the esophagus of both sexes seen at the LOAEL of 1200 ppm (57.7/69.9 mg/kg/day, M/F).

Fluensulfone showed no carcinogenic potential in rats. Dosing was considered adequate in both sexes based on the findings of decreased body weight and weight gain, slight hematological effects and microscopic findings in the lung and esophagus.

This study is classified **acceptable/guideline (fully reliable)** and satisfies the guideline requirement (OPPTS 870.4300; OECD 453) for a combined oral chronic toxicity/carcinogenicity study in the rat.

# I. MATERIAL AND METHODS

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|---|---|
| **1. Test Material** | MCW-2 TECH (fluensulfone) |
| **Description:** | Solid, yellow |
| **Lot/Batch:** | 36372130-291-PF1 |
| **Purity:** | 96.7% |
| **CAS#:** | 318290-98-1 |
| **Stability:** | Stable until 31 May 2010 |
| **2. Vehicle** | Provimi Kliba Nafag 3433 rodent maintenance diet (Provimi Kliba AG, Switzerland) |
| **3. Test Animals** | |
| **Species** | Rat |
| **Strain** | HanRcc: WIST(SPF) – males and females |
| **Age** | 5 weeks at delivery; 6-7 weeks old at start of dosing |
| **Weight** | At acclimatization: Males: 83.4 g to 127.0 g (mean 103.0 g); Females: 71.9 g to 113.7 g (mean 94.4 g) |
| **Source** | Harlan Laboratories Ltd., Switzerland |
| **Acclimation period** | 9 days |
| **Diet** | Pelleted standard Provimi Kliba Nafag 3433 rodent maintenance diet (Provimi Kliba AG, Switzerland) *ad libitum* |
| **Water** | Community tap water from Itingen *ad libitum* |
| **Housing** | In groups of 5 in Makrolon type-4 cages with wire mesh tops and sterilized standard softwood bedding (‘Lignocel’ Schill AG, Switzerland). |
| **4. Environmental conditions** | |
| **Temperature** | 22 ± 3°C. Values outside of this range occasionally occurred, usually following room cleaning, and were considered not to have any influence on the study. |
| **Humidity** | 30-70%. Values outside of this range occasionally occurred, usually following room cleaning, and were considered not to have any influence on the study. |
| **Air change** | 10-15 air changes per hour |
| **Photoperiod** | Light cycle of 12 hours light and 12 hours dark, music during the daytime light period. |

## B. STUDY DESIGN

**1. In-life dates** 20 February 2008 to 25 February 2010 (allocation A); 20 February 2008 to 22 February 2009 (allocation B).

**2. Animal Assignment and treatment**

The animals were assigned to groups using a computer-generated random algorithm. Animals in "Allocation A" (50 animals/sex/group) were treated for 104 weeks, up to a maximum of 105 weeks and 2 days (due to the high number of animals), and those in "Allocation B" (20 animals/sex/group) were treated for 52 weeks, up to a maximum of 53 weeks. Animals received the test material in the diet at nominal concentrations of 0, 30, 200, or 1200 ppm (Table 1).

**Table 1: Study Design for a Combined Chronic Toxicity/Carcinogenicity study in Rats**

| **Allocation**<br>**Dietary concentration** | | **Number of animals** | **Group 1**<br>**0 ppm** | **Group 2**<br>**30 ppm** | **Group 3**<br>**200 ppm** | **Group 4**<br>**1200 ppm** |
|---|---|---|---|---|---|---|
| | | **Daily intake (mg/kg bw/day)** | | | | |
| **Males** | **A** | 50 | 0 | 1.4 | 9.6 | 57.7 |
| | **B** | 20 | 0 | 1.6 | 11.0 | 66.3 |
| **Females** | **A** | 50 | 0 | 1.7 | 11.6 | 69.3 |
| | **B** | 20 | 0 | 1.9 | 13.1 | 75.2 |

A: Oncogenicity animals (at least 104 weeks of treatment)
B: Chronic toxicity animals (at least 52 weeks of treatment)

**3. Diet Preparation and Analysis**

Fresh batches of the feed pellets for the study were prepared every two weeks. In treatment week 43, the 2-week interval was shortened to a one week interval. Thereafter, the feed pellets were prepared every two weeks.

Fluensulfone was warmed in the original container in a water bath up to maximum of approximately 40°C until the test item was fluid. Thereafter, the whole amount of fluid test item was transferred into a glass beaker (wrapped with aluminum foil) and mixed using a magnetic stirrer on a heating plate (maximum temperature of approximately 40°C). The test item was divided into 3 aliquots.

The required amount of fluensulfone was weighed into tared glass beakers (wrapped with aluminum foil) and kept at a maximum temperature of approximately 40°C on a heating plate until use for feed preparation. The test item was mixed with microgranulated feed for each dose group. An appropriate amount of water was added to aid pelleting. The pellets were dried with air for approximately 48 to 96 hours before storage.

Control feed for the animals of group 1 were prepared similarly, but without test item.

Feed preparations were stored at room temperature (17 to 23°C) in disposable paper bags until use. They were determined to be stable for at least 24 days at room temperature in previous studies.

Stability, concentration and homogeneity of the actual test item batch in the feed were determined with the first feed preparations. The concentration and homogeneity were determined every three months thereafter with the exception of the interval between April 30, 2009 and November 12, 2009, and the interval between January 21, 2010 and February 4, 2010. In addition, the concentration was determined in the first 5 feed preparations. Double samples for the stability of the test item were assessed after 7, 14 and 21 days. One sample was stored under study conditions and one was stored protected from light.

Additional samples of the control feed from the first preparation were taken from the feed hoppers as well from the storage bag for concentration analysis.

Analyses were performed using an HPLC-method, previously developed at the performing laboratory. The diet samples were stored deep-frozen (-20°C ± 5°C) until analysis. The test item was used as analytical standard. (See "Results", section A "Analysis of Feed Preparations" for results).

### 4. Statistics

The following methods were used to analyze the water consumption, food consumption, body weight, grip strength, locomotor activity, clinical laboratory data, organ weights and ratios as well as macroscopic findings:

• If the variables were assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison of the treated groups with control groups for each sex.

• The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data cannot be assumed to follow a normal distribution.

• The Fisher's exact test

• Armitage / Cochran Trend Test for non-neoplastic lesions, if appropriate.

## C. METHODS

### 1. Observations

Observations for viability and mortality were recorded twice daily. General cage-side observations were recorded once daily during acclimatization and once daily during the treatment period. Detailed clinical observations, including palpation for tissue masses on each animal outside the home cage, were performed weekly during acclimatization and treatment periods.

**2. Bodyweight**

Body weights were recorded weekly during acclimatization and treatment weeks 1 to 14, and once every 4 weeks thereafter.

**3. Food and water consumption**

Food consumption was recorded weekly during acclimatization and treatment weeks 1 to 14, and once every 4 weeks thereafter.

The water consumption was recorded once during week 78 over a period of 24 hours, using an on-line electronic recording system consisting of a Mettler balance connected to the testing facility computer.

**4. Ophthalmoscopic examination**

Ophthalmoscopic examinations were performed on all animals during acclimatization, on all surviving allocation B animals in the control and high dose groups at the end of the treatment and on 10 allocation A animals per sex in the control and high dose groups at termination of the study. A Miroflex 2 or a Heine Beta 200 ophthalmoscope (Heine Optotechnik GmbH & Co. KG, Germany) was used.

**5. Functional Observation Battery and Motor Activity**

At week 52, relevant parameters from a modified Irwin screen test, as part of a functional observational battery (FOB) were performed on all Allocation B rats. Any abnormal findings were recorded and graded in severity. The table below summarizes the clinical and behavioral observations and their frequency.

| Category | Score | Parameter | D | P | W Weeks 1-51 | F Week 52 |
|---|---|---|---|---|---|---|
| APPEARANCE | 1-3 | Piloerection | X | X | X | X |
| | 1-3 | Salivation | X | X | X | X |
| | 1 | Hunched posture | X | X | X | X |
| MOTOR | 1-3 | Ataxia | X | X | X | X |
| | 1-3 | Tremor / twitching | X | X | X | X |
| | 1 | Prostration | X | X | X | X |
| | 1 | Circling | | X | X | X |
| | 1-3 | Spasm | | X | X | X |
| BEHAVIOR | 1-3 | Hyperactivity | X | X | X | X |
| | 1-3 | Somnolence | X | X | X | X |
| | 1-3 | Increased exploration | | X | X | X |
| | 1-3 | Reduced grooming | | X | X | X |
| | 1-3 | Vocalization | | X | X | X |
| RESPIRATION | 1 | Dyspnea | X | X | X | X |
| | 1 | Tachypnea | X | X | X | X |
| | 1 | Bradypnea | X | X | X | X |
| REFLEXES | 1 | Blink | | X | X | X |
| | 1 | Pinna | | X | X | X |
| | 1 | Iridic light reflex | | X | X | X |
| | 1 | Push-off (hind leg) | | X | X | X |
| | 1 | Pain response | | X | X | X |
| | 1 | Startle / hearing | | X | X | X |
| MISCELLANEOUS | 1-3 | Lacrimation | | X | X | X |
| | 1 | Limbs cyanotic | | X | X | X |
| | 1 | Mydriasis | | X | X | X |
| | 1 | Miosis | | X | X | X |
| | 1 | Exophthalmos | | X | X | X |
| | 1-3 | Reduced muscle tone | | X | X | X |

D: daily cage-side; P: pre-test; W: weekly; F: FOB; X: observed; 1-3: severity of finding noted. Data obtained from page 30 of the study report.

Hindlimb and forelimb grip strength measurements were performed using a push-pull strain gauge (Mecmesin, AFG 25N) at week 52.

Locomotor activity was measured quantitatively at week 52 with Activity Monitor AMS (DeMeTec GmbH, Germany). Decreased or increased activity was recorded. Activity of the animals (low-beam counts) was recorded for 10-minute intervals over a period of 60 minutes.

**6. Clinical Laboratory Investigations**

Allocation B animals: Blood and urine samples for clinical laboratory investigations (hematology, clinical chemistry, and urinalysis) were collected from all allocation B animals during weeks 13 and 26 and after 52 weeks. Blood samples were drawn from the retro-orbital plexus from all allocation B animals under light isoflurane anesthesia. The animals were fasted in metabolism cages for approximately 18 hours before blood and urine sampling but allowed access to water *ad libitum*. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms.

Allocation A animals: Blood samples for hematology (erythrocyte count, total leukocyte count, differential leukocyte count, and blood smears only) were collected under isoflurane anesthesia from all allocation A animals after weeks 78 and 104. The animals were not fasted before blood sampling. The samples were collected early in the working day to reduce biological variation caused by circadian rhythms.

Hematology:

The following hematological parameters were examined: erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, red cell volume distribution width, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hemoglobin concentration distribution width, reticulocyte count, reticulocyte maturity index, leukocyte count (total), differential leukocyte count (neutrophils, eosinophils, basophils, lymphocytes, monocytes, large unstained cells), thrombocyte count. The following parameters were examined for Allocation B animals only: prothrombin time (thromboplastin time), activated partial thromboplastin time. Blood spears were prepared from Allocation A animals but were not evaluated.

Clinical Chemistry:

The following parameters were examined for clinical chemistry investigations: glucose, urea, creatinine, total bilirubin, total cholesterol, triglycerides, phospholipids, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, glutamate dehydrogenase, alkaline phosphatase, gamma-glutamyl-transferase, creatine kinase, sodium, potassium, chloride, calcium, phosphorus, total protein, albumin, globulin, and albumin/globulin ratio.

Urinalysis:

The following parameters were determined for urinalysis: urine volume, specific gravity, colour, appearance, pH, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, erythrocytes, leukocytes.

**7. Sacrifice and pathology**

All animals were weighed and necropsied. Descriptions of all macroscopic abnormalities were recorded. All animals surviving to the end of the observation period and all animals found dead or sacrificed *in extremis* were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination.

From the first five animals of each group in allocation B, liver samples (approximately 5 g) were taken for liver enzyme determination (see point 8 below).

From the surviving first ten animals of each group in allocations A and B, the right femur and the incisors of the maxilla were used for fluoride determination (see point 8 below).

Samples of a complete list of tissues and organs were collected from all animals at necropsy and, unless otherwise indicated, fixed in neutral phosphate buffered 4% formaldehyde solution. Additional tissues (such as ear tattoo) were retained in accordance with standard operating procedures but were not processed or examined further.

From allocation B and A animals, adrenal glands, brain, epididymides, heart including auricles, kidneys, liver, ovaries, prostate including coagulating gland, spleen, testes, thymus, thyroid (including parathyroid gland, if possible) and uterus were weighed before fixation and recorded on the scheduled dates of necropsy. Relative organ weights were calculated on the basis of the body weight and brain weight. Samples were also examined from the following tissues/organs: aorta, bone (sternum, femur including joint), cecum, colon, duodenum, esophagus, eyes with optic nerve, ileum, incisors, jejunum with Peyer's patches, larynx, lungs, lymph nodes, mammary gland area, nasal cavity, pancreas, pharynx, pituitary gland, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, stomach, tongue, trachea, urinary bladder, and vagina.

Sections of all organs and tissues of the control and high-dose groups and all gross lesions from all animals were examined by the study pathologist. Organ and tissue samples taken from animals which died spontaneously or which were killed *in extremis* were evaluated similarly to those organs taken from animals of the high-dose group. Due to test item-related morphologic changes detected in the lungs and esophagus of high-dose animals, these organs were also processed and examined from the mid- and low-dose group.

**8. Additional investigations**

Liver Enzyme Determination

At scheduled necropsy after 52 weeks of treatment, additional liver samples (approximately 5 g) taken from the first 5 animals of each sex and group in allocation B (total of 40 animals) were minced in small pieces, weighed and frozen in liquid nitrogen. Liver pieces were placed in plastic bags which were labeled with the Study and Animal Number. The samples were kept at -80°C until shipment on dry ice for liver enzyme determination. Liver subcellular fractions were prepared and the following parameters determined:

• Protein content of subcellular fractions

• Microsomal cytochrome P450 content (differential spectroscopy)

• CYP1A1 (microsomal 7-ethoxyresorufin O-dealkylation)

• CYP1A2 (microsomal 7-methoxyresorufin O-dealkylation)

• CYP2B1 (microsomal 7-pentoxyresorufin O-dealkylation)

• CYP3A (microsomal testosterone 6β-hydroxylation)

• CYP4A1 (microsomal lauric acid 12-hydroxylation)

• Cytosolic glutathione S-transferase (GST, glutathione conjugation of 1-chloro-2,4-dinitrobenzol)

• Microsomal uridine diphosphoglucuronosyl-transferase (UDPGT, glucuronidation of 3-methyl-2-nitrophenol)

• Microsomal epoxide hydrolase (mEH, hydrolysis of styrene oxide)

• Cytosolic alanine aminotransferase (ALAT, transamination of ketoglutarate)

• Peroxisomal beta Oxidation

Fluoride determination

At scheduled necropsy after 52 and 104 weeks, the right femur and the incisors of the maxilla of the first surviving 10 animals per sex and group were taken for fluoride determination (allocation A: in total 80 animals; allocation B: in total 80 animals). The samples were stored at room temperature in plastic bags (Teflon® free) until shipment to the responsible study scientist. The fluoride determination was performed according to Zober [Fluorid, Bestimmung in Knochen. In: Analytische Methoden zur Prüfung gesundheitsschädlicher Arbeitsstoffe, Band 2, Analysen in biologischen Material (1. Lieferung), Senatskommision zur Prüfung gesundheitsschädlicher Arbeitsstoffe der DFG, Verlag Chemie, Weinheim (1987)] at room temperature. Analyses were performed using ion chromatography. The fluoride samples were stored frozen at -80 ± 10 °C until work up. A defined fluoride solution was used as analytical standard.

## II. RESULTS

### A. ANALYSIS OF FEED PREPARATIONS

The content of fluensulfone in 111 out of a total of 117 diet samples ranged from 82% to 107% of nominal concentration. The content of the three feed samples of dose group 2 (30 ppm) prepared on November 12, 2009 were 125% to 128% of nominal, while the three group 2 (30 ppm) diets prepared on February 4, 2010 were 139% to 141% of nominal.

The maximum variation from the mean of homogeneity samples which were taken from the top, middle and bottom of the samples ranged from 0.2% to 7.1%.

The absence of fluensulfone in the control diet was confirmed.

The stability of fluensulfone was tested in samples taken 7, 14 and 21 days after preparation and stored at room temperature. The variation values, except one, were found to be <10% from the time-zero value (range: 1.2% to 13.5%).

In conclusion, the results indicate the accurate preparation and use of fluensulfone and the control diet during this study. Diet samples were found to contain acceptable concentrations, to be homogeneously prepared, and sufficiently stable under the storage conditions.

## B. OBSERVATIONS

### 1. Clinical signs

There were no fluensulfone-related clinical signs observed during the study.

All clinical signs noted generally affected single animals of all dose groups including controls and were those commonly seen in rats of this strain and age kept under the conditions of the study. Their incidence, nature and group distribution did not distinguish treated groups from controls. The most frequent clinical signs noted were hair loss, wounds, scars and eye lesions.

### 2. Mortality

Mortality was not affected by treatment with fluensulfone (Tables 2 and 3).

**Table 2: Mortality data for animals allocated to 52-week sacrifice (Allocation B)**

| Dose (ppm) | Spontaneous death | | | | Killed *in extremis* | | | | Planned necropsy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Males | | Females | | Males | | Females | | Males | | Females | |
| **0** | 0 | 0% | 2 | 10% | 0 | 0% | 0 | 0% | 20 | 100% | 18 | 90% |
| **30** | 0 | 0% | 0 | 0% | 0 | 0% | 0 | 0% | 20 | 100% | 20 | 100% |
| **200** | 0 | 0% | 0 | 0% | 0 | 0% | 0 | 0% | 20 | 100% | 20 | 100% |
| **1200** | 0 | 0% | 0 | 0% | 0 | 0% | 0 | 0% | 20 | 100% | 20 | 100% |

Data obtained from page 40 of the study report.

**Table 3: Mortality data for animals allocated to 104-week sacrifice (Allocation A)**

| Dose (ppm) | Spontaneous death | | | | Killed *in extremis* | | | | Planned necropsy | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Males | | Females | | Males | | Females | | Males | | Females | |
| **0** | 3 | 6% | 2 | 4% | 8 | 16% | 12 | 24% | 39 | 78% | 36 | 72% |
| **30** | 4 | 8% | 2 | 4% | 4 | 8% | 12 | 24% | 42 | 84% | 36 | 72% |
| **200** | 8 | 16% | 10 | 20% | 7 | 14% | 7 | 14% | 35 | 70% | 33 | 66% |
| **1200** | 4 | 8% | 4 | 8% | 8 | 16% | 8 | 16% | 38 | 76% | 38 | 76% |

Data obtained from page 39 of the study report

### 3. FOB, Grip Strength and Locomotor Activity

Locomotor activity was assessed at week 52 in Allocation B animals. Overall mean locomotor activity was decreased by 14% in high dose males. Decreases in activity were noted at the 0-10 minute interval, as well as at the 30-40 and 50-60 minute intervals (Table 4). In females, total overall activity was increased by 20% at the high dose. This increase is largely due to a 70% increase at the 50-60 minute interval. Overall, values were variable throughout the assessment period. No treatment-related effects were recorded during the FOB or grip strength assessments.

**Table 4: Mean locomotor activity per group (± S.D.) at 52 weeks (Allocation B):**

| **Interval** | **Control n = 20** | **30 ppm n = 20** | **200 ppm n = 20** | **1200 ppm n = 20** |
|---|---|---|---|---|
| **MALES** | | | | |
| 0-10 minutes | 240 ± 107 | 221 ± 80 (↓8%) | 199 ± 114 (↓17%) | 186 ± 107 (↓23%) |
| 10-20 minutes | 66 ± 80 | 72 ± 36 | 71 ± 64 | 80 ± 60 (↑21%) |
| 20-30 minutes | 49 ± 48 | 49 ± 46 | 67 ± 65 (↑37%) | 50 ± 33 |
| 30-40 minutes | 39 ± 54 | 45 ± 52 | 70 ± 50 (↑79%) | 28 ± 25 (↓28%) |
| 40-50 minutes | 40 ± 53 | 53 ± 42 | 43 ± 54 | 41 ± 42 |
| 50-60 minutes | 39 ± 47 | 30 ± 46 | 17 ± 25 (↓56%) | 25 ± 33 (↓36%) |
| Total | 474 ± 238 | 470 ± 215 | 467 ± 196 | 410 ± 218 (↓14%) |
| **Interval** | **Control n = 18** | **30 ppm n = 20** | **200 ppm n = 20** | **1200 ppm n = 20** |
| **FEMALES** | | | | |
| 0-10 minutes | 237 ± 76 | 224 ± 80 | 237 ± 107 | 254 ± 93 |
| 10-20 minutes | 110 ± 69 | 86 ± 46 | 110 ± 92 | 113 ± 66 |
| 20-30 minutes | 83 ± 54 | 68 ± 59 | 90 ± 106 | 84 ± 56 |
| 30-40 minutes | 69 ± 79 | 60 ± 73 | 52 ± 66 | 77 ± 62 |
| 40-50 minutes | 60 ± 56 | 59 ± 56 | 47 ± 63 | 41 ± 50 |
| 50-60 minutes | 59 ± 58 | 65 ± 69 | 68 ± 65 | 100 ± 84 (↑70%) |
| Total | 556 ± 311 | 562 ± 256 | 604 ± 389 (↑9%) | 669 ± 255 (↑20%) |

Data obtained from pages 215-218 of the study report. ( ) = % different from controls, calculated by the reviewer.

## C. BODYWEIGHT AND BODYWEIGHT GAINS

Absolute mean body weight and body weight gain were decreased in a dose-dependent manner in males treated at 200 and 1200 ppm, although not always achieving statistical significance at 200 ppm (Tables 5 and 6). Mean body weight of males treated at 1200 ppm was 11% lower than controls on day 715, thus indicating that a Maximum Tolerated Dose (MTD) was reached in this study. In high dose females, body weight was decreased during the last 11 weeks of the study in the range of 3 to 6%. These decreases did not reach statistical significance. Body weight gain was statistically significantly reduced in females at 1200 ppm from week 2 onwards. Overall body weight gain was decreased by 6% in 200 ppm males, by 14% in 1200 ppm males, and by 10% in 1200 ppm females.

**Table 5: Mean (± S.D.) Body Weight and Overall Body Weight Gain (g)**

| Study Day | Control | 30 ppm | 200 ppm | 1200 ppm |
|---|---|---|---|---|
| **MALES** | | | | |
| Day 1 | 166 ± 15 | 164 ± 13 | 163 ± 14 | 167 ± 14 |
| Day 15 | 257 ± 22 | 253 ± 18 | 250 ± 19 | 249* ± 17 (↓3%) |
| Day 85 | 431 ± 43 | 424 ± 37 | 411** ± 39 (↓5%) | 408** ± 35 (↓5%) |
| Day 239 | 542 ± 61 | 535 ± 54 | 517* ± 50 (↓5%) | 510** ± 48 (↓6%) |
| Day 603 | 688 ± 88 | 677 ± 71 | 639* ± 84 (↓7%) | 624** ± 82 (↓9%) |
| Day 715 | 703 ± 87 | 691 ± 78 | 668 ± 93 (↓5%) | 628** ± 99 (↓11%) |
| BWG | 537 | 527 | 505 (↓6%) | 461 (↓14%) |
| **FEMALES** | | | | |
| Day 1 | 131 ± 10 | 132 ± 10 | 132 ± 10 | 133 ± 11 |
| Day 15 | 172 ± 12 | 175 ± 12 | 174 ± 13 | 171 ± 13 |
| Day 85 | 246 ± 17 | 248 ± 19 | 244 ± 21 | 244 ± 19 |
| Day 239 | 284 ± 25 | 290 ± 29 | 286 ± 37 | 277 ± 28 (↓3%) |
| Day 603 | 390 ± 53 | 396 ± 58 | 393 ± 62 | 370 ± 58 (↓5%) |
| Day 715 | 402 ± 53 | 408 ± 64 | 404 ± 63 | 378 ± 61 (↓6%) |
| BWG | 271 | 276 | 272 | 245 (↓10%) |

Data obtained from pages 320-327 of the study report. ( ) = % difference from control (calculated by the reviewer). * $p < 0.05$; ** $p < 0.01$. N=70/sex/dose at start of study. BWG = overall body weight gain, calculated by the reviewer from the mean body weight values on days 715 and 1 (not analyzed statistically).

**Table 6: Mean (± S.D.) Body Weight Gain (% of initial bw)**

| Study Day | Control | 30 ppm | 200 ppm | 1200 ppm |
|---|---|---|---|---|
| **MALES** | | | | |
| Day 1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Day 15 | 55.2 ± 4.7 | 54.0 ± 4.1 | 53.6 ± 4.9 | 49.1** ± 5.2 |
| Day 85 | 160.7 ± 19.1 | 158.7 ± 15.8 | 152.7* ± 18.7 | 144.7** ± 15.5 |
| Day 239 | 228.3 ± 29.5 | 226.5 ± 25.2 | 217.9 ± 26.8 | 205.9** ± 23.9 |
| Day 603 | 315.4 ± 43.9 | 316.0 ± 35.8 | 293.8* ± 46.8 | 273.7** ± 40.8 |
| Day 715 | 325.1 ± 48.8 | 324.6 ± 44.7 | 313.9 ± 47.5 | 275.2** ± 51.2 (↓15%) |
| **FEMALES** | | | | |
| Day 1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Day 15 | 31.2 ± 4.6 | 32.6 ± 5.1 | 31.6 ± 4.6 | 28.4** ± 4.9 |
| Day 85 | 88.0 ± 9.8 | 88.4 ± 11.5 | 85.0 ± 11.3 | 83.6* ± 10.7 |
| Day 239 | 117.7 ± 16.8 | 120.0 ± 20.3 | 116.7 ± 23.5 | 108.4* ± 17.4 |
| Day 603 | 198.5 ± 36.4 | 200.2 ± 41.9 | 195.3 ± 41.1 | 178.6 ± 42.1 |
| Day 715 | 208.6 ± 37.1 | 208.3 ± 40.6 | 202.2 ± 39.7 | 181.4* ± 42.9 (↓13%) |

Data obtained from pages 330 – 337 of the study report. * $p < 0.05$; ** $p < 0.01$. N=70/sex/dose at start of study.

## D. FOOD AND WATER CONSUMPTION

Slightly lower absolute (g/animal/day) and relative (g/kg bw/day) food consumption was recorded in both sexes at all dose levels during the first 1-2 weeks of dosing (Table 7). This effect is commonly seen in feeding studies, is caused by the change of feed (e.g. due to different taste of the feed) and is not considered to be of toxicological relevance.

Lower absolute food consumption was recorded in males treated at 1200 ppm during the first 4 weeks of treatment. Afterwards statistically significantly lower absolute food consumption was recorded at several intervals. However, the lower absolute food consumption was likely caused by lower body weights, as relative food consumption in the same animals was similar to, or higher than in the controls. The mean of means for food consumption for each dose group was comparable for both sexes, indicating overall similar food consumption.

The 200 ppm group males occasionally had statistically significant reduced absolute food consumption; however, the relative food consumption values were not affected. Therefore these changes were not considered adverse.

Relative water consumption was statistically significantly increased during week 78 in males treated at 1200 ppm (15%). Relative water consumption was also increased in females of this dose group (7%), although the increase was slight and not statistically significant (Table 8).

**Table 7a: Absolute (g/animal/day) and relative (g/kg bw/day) mean (± S.D.) food consumption in males**

| Study Days | | Control | 30 ppm | 200 ppm | 1200 ppm |
|---|---|---|---|---|---|
| 1 – 8 | absolute | 19.9 ± 0.9 | 19.0 ± 0.8** (↓4%) | 18.9 ± 0.7** (↓5%) | 18.4 ± 0.7** (↓7%) |
| | relative | 93.3 ± 2.6 | 89.4 ± 2.0** (↓4%) | 89.7** ± 3.0 (↓4%) | 87.8 ± 2.4** (↓6%) |
| 8 – 15 | absolute | 22.2 ± 0.8 | 21.4 ± 0.9* (↓4%) | 21.3 ± 0.6* (↓4%) | 21.2 ± 0.8** (↓4%) |
| | relative | 86.4 ± 1.7 | 84.7 ± 2.0 | 85.2 ± 2.4 | 85.4 ± 2.5 |
| 15 – 22 | absolute | 21.8 ± 0.8 | 21.4 ± 1.2 (↓2%) | 21.4 ± 0.7 (↓2%) | 21.3 ± 0.8 (↓2%) |
| | relative | 75.2 ± 2.2 | 75.0 ± 2.7 | 76.2 ± 2.8 | 76.5 ± 2.3 |
| 22 – 29 | absolute | 23.3 ± 0.9 | 22.4 ± 1.0 (↓3%) | 22.6 ± 1.8 (↓3%) | 21.4** ± 0.7 (↓8%) |
| | relative | 73.2 ± 2.4 | 71.9 ± 1.7 | 73.8 ± 4.8 | 70.2 ± 1.9* (↓4%) |
| 176 – 183 | absolute | 21.7 ± 0.9 | 21.9 ± 1.0 | 21.6 ± 1.0 | 21.4 ± 0.7 |
| | relative | 42.1 ± 1.5 | 42.9 ± 1.9 | 43.4 ± 1.6 | 43.6 ± 1.5 |
| 372 – 379 | absolute | 22.2 ± 1.1 | 22.2 ± 0.8 | 21.4 ± 1.2 (↓4%) | 21.0 ± 1.2* (↓5%) |
| | relative | 35.7 ±1.0 | 36.4 ± 1.4 | 36.5 ± 1.1 | 36.0 ± 1.2 |
| 568 – 575 | absolute | 22.8 ± 1.1 | 22.6 ± 1.0 | 20.7 ± 1.3* (↓9%) | 20.8 ± 2.7* (↓9%) |
| | relative | 34.1 ± 1.6 | 34.4 ± 1.3 | 33.4 ± 1.8 | 34.5 ± 6.6 |
| 708 – 715 | absolute | 20.6 ± 1.8 | 20.9 ± 1.4 | 20.0 ± 1.8 (↓3%) | 19.8 ± 1.8 (↓4%) |
| | relative | 29.4 ± 2.1 | 29.8 ± 1.5 | 30.1 ± 2.5 | 32.1 ± 3.0* (↑9%) |

Data obtained from pages 281 – 298 of the data report. n = 14 from the beginning of the study until reading for days 344 – 351. n = 10 for reading on days 372-379 to study termination. * $p < 0.05$; ** $p < 0.01$; ( ) = % difference from control, calculated the by reviewer.

**Table 7b: Absolute (g/animal/day) and relative (g/kg bw/day) mean (± S.D.) food consumption in females**

| Study Days | | Control | 30 ppm | 200 ppm | 1200 ppm |
|---|---|---|---|---|---|
| 1 – 8 | absolute | 14.7 ± 0.8 | 14.0 ± 0.5* (↓5%) | 13.9 ± 0.7** (↓5%) | 13.4 ± 0.6** (↓9%) |
| | relative | 95.4 ± 4.4 | 90.8 ± 1.9** (↓5%) | 89.9 ± 3.7** (↓6%) | 86.9 ± 2.3** (↓9%) |
| 8 – 15 | absolute | 14.9 ± 0.5 | 15.0 ± 0.5 | 15.0 ± 0.7 | 14.7 ± 0.6 |
| | relative | 86.7 ± 1.4 | 86.1 ± 2.3 | 86.2 ± 3.1 | 86.2 ± 2.7 |
| 15 – 22 | absolute | 14.6 ± 0.5 | 15.0 ± 0.6 (↑3%) | 15.3 ± 1.0* (↑5%) | 15.2 ± 0.7 (↑4%) |
| | relative | 77.8 ± 1.5 | 79.7 ± 2.0 | 82.3 ± 3.5** (↑6%) | 82.2 ± 2.7** (↑6%) |
| 22 – 29 | absolute | 15.4 ± 0.7 | 15.6 ± 0.8 | 15.7 ± 0.8 | 15.3 ± 0.7 |
| | relative | 77.2 ± 2.4 | 78.3 ± 2.5 | 79.0 ± 3.2 | 77.0 ± 3.4 |
| 176 – 183 | absolute | 13.7 ± 1.1 | 13.9 ± 1.3 | 14.3 ± 1.0 (↑4%) | 14.2 ± 0.9 (↑4%) |
| | relative | 51.5 ± 4.0 | 51.4 ± 3.7 | 53.0 ± 3.2 | 53.5 ± 3.9 |
| 372 – 379 | absolute | 15.8 ± 0.8 | 15.9 ± 0.8 | 15.7 ± 2.3 | 15.7 ± 0.9 |
| | relative | 47.9 ± 2.7 | 47.5 ± 2.2 | 46.7 ± 4.8 | 48.9 ± 2.2 |
| 568 – 575 | absolute | 16.6 ± 2.0 | 17.0 ± 1.2 | 16.7 ± 1.2 | 15.5 ± 1.5 (↓7%) |
| | relative | 44.8 ± 4.2 | 44.1 ± 2.4 | 43.9 ± 4.2 (↓2%) | 43.1 ± 5.6 (↓4%) |
| 708 – 715 | absolute | 14.8 ± 1.5 | 15.2 ± 1.2 | 14.6 ± 1.6 | 13.9 ± 1.6 (↓6%) |
| | relative | 37.1 ± 3.0 | 37.0 ± 4.1 | 36.3 ± 3.7 | 36.7 ± 4.2 |

Data obtained from pages 281 – 298 of the data report. n = 14 from the beginning of the study until reading for days 344 – 351. n = 10 for reading on days 372-379 to study termination. * $p < 0.05$; ** $p < 0.01$; ( ) = % difference from control, calculated by the reviewer.

**Table 8: Mean water consumption (g/animal/day and mg/kg bw/day)**

| | Control n = 10 | 30 ppm n = 10 | 200 ppm n = 10 | 1200 ppm n = 10 |
|---|---|---|---|---|
| **MALES** | | | | |
| g/animal/day | 27.2 ± 2.4 | 25.8 ± 2.6 | 26.8 ± 3.5 | 28.7 ± 4.0 (↑5%) |
| mg/kg bw/day | 40.7 ± 3.8 | 39.3 ± 4.4 | 42.8 ± 7.3 | 46.9* ± 6.1 (↑15%) |
| **FEMALES** | | | | |
| g/animal/day | 26.3 ± 4.9 | 27.3 ± 3.5 | 27.0 ± 5.1 | 26.8 ± 3.6 |
| mg/kg bw/day | 70.7 ± 12.9 | 71.3 ± 7.3 | 71.7 ± 17.6 | 75.9 ± 10.4 (↑7%) |

Data obtained from pages 300-304 of the study report. ( ) = % difference from control, calculated by the reviewer. * $p < 0.05$; ** $p < 0.01$.

## E. OPHTHALMOSCOPY

No ophthalmoscopic findings of toxicological relevance were recorded.

## F. BLOOD ANALYSIS

### 1. Hematological findings

The following changes noted at 52 weeks were considered to be related to treatment with fluensulfone (Tables 9 and 10):

- Increased absolute red blood cell count (RBC) in males at 1200 ppm (5%);

- Decreased hemoglobin concentration (Hb) in females at 1200 ppm (3%);

- Decreased mean corpuscular hemoglobin (MCH) in males at 1200 ppm (5%);

- Decreased mean corpuscular hemoglobin concentration (MCHC) in males (2%) and females (1%) at 1200 ppm;

- Dose dependent decreased neutrophil count (Neut) in all female dose groups (19, 23, 38%), attaining statistical significance at 1200 ppm;

- Decreased lymphocytes in 1200 ppm females (17%);

- Decreased prothrombin time (PT) in males at 200 and 1200 ppm (5% and 9%, respectively), and increased partial prothrombin time (PTT) in males at 1200 ppm (10%);

- LUC (large unstained cell) counts were increased in 1200 ppm males (33%);

- White blood cell counts (WBC) counts were decreased in a dose-dependent manner in all female treatment groups at week 52 (10%, 13%, and 24% at 30, 200 and 1200 ppm, respectively).

In most of the parameters similar changes were also recorded after 13 and 26 weeks.

The following changes noted at 78 and/or 104 weeks were considered to be related to treatment with fluensulfone:

- Decreased hemoglobin concentration (Hb) in females at 1200 ppm (2%);

- Increased absolute RBC in males at 1200 ppm (4%);

- Decreased MCH in males at 1200 ppm (3%);

- Decreased MCHC in males at all doses at 78 weeks (1-3%) and at 1200 ppm (1%) at 104 weeks, as well as in females at 200 and 1200 ppm (1% and 2%, respectively);

- Decreased hemoglobin concentration distribution width (HDW) in females at 200 and 1200 ppm (5% and 6%, respectively), as well as a slight decrease in females at 30 ppm at week 78 only which is not considered toxicologically relevant;

- Increased neutrophil counts (Neut) in females (53%) at 1200 ppm;

- Decreased lymphocyte counts (Lymph) of 19% in females in both 200 and 1200 ppm dose groups at week 78;

- Increased eosinophil (EOS) values in 1200 ppm males (50%).

At week 104, one control male (animal #12) demonstrated very high values for several white blood cell parameters and means were re-calculated after excluding these outlying values (values of 165 G/L for total WBC, 19.76 G/L for large unstained cells, and 140 G/L for lymphocytes).

At 78 weeks, males in the 200 ppm dose group also exhibited a statistically significant increase in mean red blood cell count when compared to controls, but this finding is considered to be spurious as changes were not observed at any other time point.

Changes in several other parameters achieving statistical significance were considered unrelated to treatment since they lacked a dose-relationship and/or were clearly within the historical control range.

## Table 9a: Selected (± S.D.) mean red blood cell parameters – Male rats

| Dose (ppm) | RBC (T/L) | Hb (mmol/L) | MCH (fmol) | MCHC (mmol/L) | HDW (mmol/L) |
|---|---|---|---|---|---|
| **Week 13 (allocation B animals)** | | | | | |
| 0 | 8.80 ± 0.51 | 10.4 ± 0.4 | 1.18 ± 0.06 | 23.34 ± 0.49 | 1.83 ± 0.11 |
| 30 | 8.77 ± 0.38 | 10.3 ± 0.4 | 1.17 ± 0.04 | 23.65 ± 0.31* | 1.91 ± 0.09 |
| 200 | 8.80 ± 0.37 | 10.2 ± 0.3 | 1.17 ± 0.04 | 23.29 ± 0.30 | 1.84 ± 0.13 |
| 1200 | 9.06 ± 0.32 (↑3%) | 10.3 ± 0.3 | 1.13 ± 0.01** (↓4%) | 22.83 ± 0.46** (↓2%) | 1.78 ± 0.14 |
| **Week 26 (allocation B animals)** | | | | | |
| 0 | 8.81 ± 0.49 | 9.7 ± 0.3 | 1.10 ± 0.06 | 22.43 ± 0.47 | 1.69 ± 0.11 |
| 30 | 8.94 ± 0.35 | 9.7 ± 0.3 | 1.09 ± 0.04 | 22.37 ± 0.32 | 1.74 ± 0.11 |
| 200 | 8.81 ± 0.28 | 9.5 ± 0.3 | 1.08 ± 0.04 | 21.15 ± 0.32* | 1.69 ± 0.15 |
| 1200 | 9.22 ± 0.41** (↑5%) | 9.7 ± 0.3 | 1.06 ± 0.04** (↓4%) | 21.88 ± 0.31** (↓2%) | 1.60 ± 0.13 |
| **Week 52 (allocation B animals)** | | | | | |
| 0 | 8.89 ± 0.53 | 10.1 ± 0.3 | 1.14 ± 0.07 | 23.10 ± 0.43 | 1.81 ± 0.14 |
| 30 | 9.01 ± 0.43 | 10.1 ± 0.4 | 1.13 ± 0.01 | 23.46 ± 0.37** | 1.86 ± 0.12 |
| 200 | 9.11 ± 0.39 | 10.2 ± 0.3 | 1.12 ± 0.05 | 23.04 ± 0.38 | 1.79 ± 0.15 |
| 1200 | 9.34 ± 0.42** (↑5%) | 10.1 ± 0.3 | 1.08 ± 0.04** (↓5%) | 22.59 ± 0.32** (↓2%) | 1.74 ± 0.15 |
| **Week 78 (allocation A animals)** | | | | | |
| 0 | 8.95 ± 0.40 | 9.8 ± 0.3 | 1.09 ± 0.05 | 21.44 ±0.37 | 1.58 ± 0.08 |
| 30 | 9.05 ± 0.40 | 9.8 ± 0.4 | 1.08 ± 0.04 | 21.11 ± 0.39** (↓1%) | 1.57 ± 0.08 |
| 200 | 9.16 ± 0.41* | 9.8 ± 0.3 | 1.07 ± 0.05 | 20.95 ± 0.45** (↓2%) | 1.56 ± 0.12 |
| 1200 | 9.13 ± 0.45 (↑2%) | 9.6 ± 0.4 | 1.06 ± 0.04** (↓3%) | 20.88 ± 0.30** (↓3%) | 1.53 ± 0.12 |
| **Week 104 (allocation A animals)** | | | | | |
| 0 | 8.54 ± 0.60 | 9.4 ± 0.5 | 1.10 ± 0.06 | 21.42 ± 0.39 | 1.63 ± 0.09 |
| 30 | 8.46 ± 0.77 | 9.4 ± 0.8 | 1.11 ± 0.05 | 21.47 ± 0.34 | 1.62 ± 0.10 |
| 200 | 8.65 ± 0.54 | 9.6 ± 0.4 | 1.11 ± 0.05 | 21.43 ± 0.42 | 1.62 ± 0.13 |
| 1200 | 8.91 ± 0.46* (↑4%) | 9.6 ± 0.4 | 1.07 ± 0.05 (↓3%) | 21.20 ± 0.38* (↓1%) | 1.62 ± 0.15 |

T: tera ($10^{12}$); m: milli ($10^{-3}$); mol: mole; f: femto ($10^{-15}$); *: significant at 5%; **: significant at 1%; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 354-368 of the study report. No standard deviation values were provided in the study report and thus were calculated by the reviewer. Weeks 13, 26 and 52, n = 20. Weeks 78 and 104, n = 50.

## Table 9b: Selected mean (± S.D.) red blood cell parameters – Female rats

| Dose (ppm) | RBC (T/L) | Hb (mmol/L) | MCH (fmol) | MCHC (mmol/L) | HDW (mmol/L) |
|---|---|---|---|---|---|
| **Week 13 (allocation B animals)** | | | | | |
| 0 | 8.01 ± 0.23 | 9.9 ± 0.2 | 1.24 ± 0.04 | 23.27 ± 0.30 | 1.53 ± 0.10 |
| 30 | 7.80 ± 0.40 | 9.7 ± 0.3 | 1.25 ± 0.05 | 23.51 ± 0.35* | 1.56 ± 0.09 |
| 200 | 7.91 ± 0.39 | 9.8 ± 0.3 | 1.24 ± 0.04 | 23.15 ± 0.26 | 1.49 ± 0.09 |
| 1200 | 7.90 ± 0.37 | 9.6 ± 0.4* (↓3%) | 1.22 ± 0.03 | 22.90 ± 0.29** (↓2%) | 1.51 ± 0.11 |
| **Week 26 (allocation B animals)** | | | | | |
| 0 | 8.04 ± 0.34 | 9.3 ± 0.3 | 1.16 ± 0.03 | 22.22 ± 0.42 | 1.40 ± 0.10 |
| 30 | 7.97 ± 0.40 | 9.3 ± 0.3 | 1.17 ± 0.05 | 22.33 ± 0.37 | 1.41 ± 0.09 |
| 200 | 7.97 ± 0.34 | 9.3 ± 0.3 | 1.17 ± 0.04 | 22.28 ± 0.27 | 1.35 ± 0.10 |
| 1200 | 7.93 ± 0.30 | 9.1 ± 0.3* (↓2%) | 1.15 ± 0.03 | 21.93 ± 0.29* (↓1%) | 1.36 ± 0.09 |
| **Week 52 (allocation B animals)** | | | | | |
| 0 | 8.02 ± 0.41 | 9.8 ± 0.4 | 1.22 ± 0.04 | 23.25 ± 0.42 | 1.45 ± 0.10 |
| 30 | 8.15 ± 0.37 | 10.0 ± 0.3 | 1.22 ± 0.06 | 23.49 ± 0.42 | 1.47 ± 0.09 |
| 200 | 8.14 ± 0.37 | 9.9 ± 0.3 | 1.21 ± 0.05 | 23.13 ± 0.43 | 1.40 ± 0.10 |
| 1200 | 7.79 ± 0.64 | 9.5 ± 0.6* (↓3%) | 1.22 ± 0.06 | 22.95 ± 0.37 (↓1%) | 1.41 ± 0.15 |
| **Week 78 (allocation A animals)** | | | | | |
| 0 | 7.90 ± 0.42 | 9.3 ± 0.3 | 1.18 ± 0.05 | 21.57 ± 0.4 | 1.46 ± 0.09 |
| 30 | 7.77 ± 0.39 | 9.2 ± 0.3 | 1.19 ± 0.03 | 21.63 ± 0.45 | 1.41 ± 0.08* (↓3%) |
| 200 | 7.95 ± 0.33 | 9.2 ± 0.3 | 1.16 ± 0.04 | 21.33 ± 0.41* (↓1%) | 1.36 ± 0.07** (↓7%) |
| 1200 | 7.76 ± 0.44 | 9.0 ± 0.3** (↓3%) | 1.16 ± 0.05 | 21.23 ± 0.48** (↓2%) | 1.37 ± 0.10** (↓6%) |
| **Week 104 (allocation A animals)** | | | | | |
| 0 | 7.61 ± 0.43 | 9.1 ± 0.3 | 1.20 ± 0.05 | 21.98 ± 0.36 | 1.46 ± 0.08 |
| 30 | 7.47 ± 0.44 | 9.0 ± 0.4 | 1.20 ± 0.04 | 21.95 ± 0.36 | 1.45 ± 0.11 |
| 200 | 7.70 ± 0.33 | 9.1 ± 0.3 | 1.19 ± 0.04 | 21.70 ± 0.41** (↓1%) | 1.39 ± 0.11* (↓5%) |
| 1200 | 7.51 ± 0.54 | 8.9 ± 0.5** (↓2%) | 1.19 ± 0.07 | 21.57 ± 0.34** (↓2%) | 1.37 ± 0.12** (↓6%) |

T: tera ($10^{12}$); m: milli ($10^{-3}$); mol: mole; f: femto ($10^{-15}$); *: significant at 5%; **: significant at 1%; nd: not determined; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 354-368 of the study report. Data obtained from pages 354-368 of the study report. No standard deviation values were provided in the study report and thus were calculated by the reviewer. Weeks 13, 26 and 52, n = 20. Weeks 78 and 104, n = 50.

## Table 10a: Selected mean (± S.D.) white blood cell and clotting parameters – Male rats

| Dose (ppm) | Neu (G/L) | Lymph (G/L) | PT (a) | PTT (sec) | LUC (G/L) | WBC (G/L) |
|---|---|---|---|---|---|---|
| **Week 13 (allocation B animals)** | | | | | | |
| 0 | 1.38 ± 0.36 | 4.62 ± 0.85 | 0.92 ± 0.05 | 24.4 ± 9.8 | 0.06 ± 0.02 | 6.36 ± 1.14 |
| 30 | 1.47 ± 0.44 | 4.57 ± 1.37 | 0.91 ± 0.03 | 23.8 ± 1.6* | 0.06 ± 0.02 | 6.36 ± 1.72 |
| 200 | 1.53 ± 0.41 | 5.50 ± 1.12* | 0.92 ± 0.08 | 23.6 ± 1.9 | 0.07 ± 0.03 | 7.39 ± 1.40 |
| 1200 | 1.28 ± 0.38 | 5.34 ± 0.91 | 0.87 ± 0.07 (↓5%) | 26.0 ± 1.8** (↑7%) | 0.08 ± 0.03* (↑33%) | 7.00 ± 1.18 |
| **Week 26 (allocation B animals)** | | | | | | |
| 0 | 1.29 ± 0.40 | 3.84 ± 0.72 | 0.87 ± 0.03 | 25.3 ± 1.4 | 0.07 ± 0.03 | 5.42 ± 1.01 |
| 30 | 1.56 ± 0.35 | 4.31 ± 0.84 | 0.89 ± 0.03 | 26.1 ± 1.3 | 0.08 ± 0.03 | 6.22 ± 1.08 |
| 200 | 1.52 ± 0.34 | 3.94 ± 0.96 | 0.86 ± 0.04 | 26.8 ± 2.3* | 0.07 ± 0.02 | 5.76 ± 1.18 |
| 1200 | 1.50 ± 0.60 | 4.32 ± 0.93 | 0.81 ± 0.07** (↓7%) | 27.8 ± 2.1** (↑10%) | 0.09 ± 0.03* (↑29%) | 6.18 ± 1.33 |
| **Week 52 (allocation B animals)** | | | | | | |
| 0 | 1.51 ± 0.72 | 3.19 ± 0.57 | 0.86 ± 0.04 | 24.5 ± 2.2 | 0.06 ± 0.04 | 5.07 ± 1.17 |
| 30 | 1.97 ± 0.61 | 3.76 ± 1.07 | 0.85 ± 0.03 | 25.0 ± 1.5 | 0.07 ± 0.03 | 6.16 ± 1.45* |
| 200 | 2.01 ± 0.63* | 3.79 ± 0.93 | 0.82 ± 0.04* (↓5%) | 25.1 ± 1.7 | 0.07 ± 0.02 | 6.21 ± 1.35* |
| 1200 | 1.47 ± 0.45 | 3.78 ± 0.57 | 0.78 ± 0.08** (↓9%) | 27.0 ± 2.2** (↑10%) | 0.08 ± 0.03* (↑33%) | 5.65 ± 0.99 |
| **Dose (ppm)** | **Neu (G/L)** | **Lymph (G/L)** | **Eos (G/L)** | **Mono (G/L)** | **LUC (G/L)** | **WBC (G/L)** |
| **Week 78 (allocation A animals)** | | | | | | |
| 0 | 1.79 ± 0.86 | 4.20 ± 0.97 | 0.13 ± 0.05 | 0.20 ± 0.09 | 0.07 ± 0.04 | 6.44 ± 1.66 |
| 30 | 1.69 ± 0.43 | 3.86 ± 0.72 | 0.12 ± 0.05 | 0.18 ± 0.05 | 0.06 ± 0.02* | 5.94 ± 1.02 |
| 200 | 1.72 ± 0.47 | 4.16 ± 0.84 | 0.12 ± 0.05 | 0.19 ± 0.06 | 0.06 ± 0.02 | 6.29 ± 1.15 |
| 1200 | 2.02 ± 0.63 | 4.04 ± 0.76 | 0.14 ± 0.06 | 0.19 ± 0.06 | 0.07 ± 0.02 | 6.49 ± 1.22 |
| **Week 104 (allocation A animals)** | | | | | | |
| 0 | 2.20 ± 2.39 | 7.35 ± 21.85 (without outlier: 3.89 ± 1.43) | 0.10 ± 0.05 | 0.20 ± 0.09 | 0.58 ± 3.00 (without outlier: 0.07 ± 0.08 ) | 10.45 ± 25.6 (without outlier: 6.39 ± 3.41) |
| 30 | 1.90 ± 1.43 | 3.42 ± 0.70 | 0.12 ± 0.08 | 0.18 ± 0.06 | 0.04 ± 0.02 | 5.69 ± 1.58 |
| 200 | 1.77 ± 0.50 | 3.51 ± 0.61 | 0.11 ± 0.04 | 0.17 ± 0.06 | 0.05 ± 0.03 | 5.61 ± 0.93 |
| 1200 | 2.39 ± 1.72 | 4.11 ± 0.99 | 0.15 ± 0.06** (↑50%) | 0.23 ± 0.11 (↑15%) | 0.06 ± 0.03 | 6.95 ± 2.39 |

G: giga ($10^9$); a: Clotting assay, thromboplastin from rabbit brain tissue, results as ratio of normal activity; *: significant at 5%; **: significant at 1%; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 354-368 of the study report. No standard deviation values were provided in the study report and thus were calculated by the reviewer. Weeks 13, 26 and 52, n = 20. Weeks 78 and 104, n = 50.

## Table 10b: Selected mean (± S.D.) white blood cell and clotting parameters – Female rats

| Dose (ppm) | Neu (G/L) | Lymph (G/L) | PT (a) | PTT (sec) | LUC (G/L) | WBC (G/L) |
|---|---|---|---|---|---|---|
| **Week 13 (allocation B animals)** | | | | | | |
| 0 | 0.71 ± 0.23 | 3.03 ± 0.75 | 0.94 ± 0.04 | 29.2 ± 4.5 | 0.05 ± 0.02 | 3.96 ± 0.81 |
| 30 | 0.72 ± 0.21 | 2.23 ± 0.74* | 0.92 ± 0.06 | 30.0 ± 3.6 | 0.03 ± 0.01** | 3.11 ± 0.78* |
| 200 | 0.76 ± 0.21 | 2.79 ± 0.94 | 0.95 ± 0.07 | 26.8 ± 4.2 | 0.04 ± 0.02 | 3.74 ± 1.04 |
| 1200 | 0.80 ± 0.17 | 2.70 ± 1.08 | 0.97 ± 0.04* | 26.1 ± 3.3 | 0.04 ± 0.02* | 3.71 ± 1.22 |
| **Week 26 (allocation B animals)** | | | | | | |
| 0 | 0.79 ± 0.27 | 2.01 ± 0.54 | 0.90 ± 0.03 | 35.2 ± 5.4 | 0.04 ± 0.02 | 2.97 ± 0.59 |
| 30 | 0.74 ± 0.33 | 2.55 ± 0.83* | 0.89 ± 0.04 | 36.5 ± 7.1 | 0.05 ± 0.03 | 3.49 ± 0.99 |
| 200 | 0.79 ± 0.24 | 2.20 ± 0.46 | 0.90 ± 0.04 | 34.2 ± 5.5 | 0.05 ± 0.03 | 3.18 ± 0.64 |
| 1200 | 0.65 ± 0.21 | 1.95 ± 0.60 | 0.89 ± 0.04 | 32.6 ± 4.6 | 0.05 ± 0.02 | 2.79 ± 0.71 |
| **Week 52 (allocation B animals)** | | | | | | |
| 0 | 0.95 ± 0.40 | 2.27 ± 0.60 | 0.86 ± 0.05 | 29.3 ± 5.3 | 0.04 ± 0.01 | 3.46 ± 0.85 |
| 30 | 0.77 ± 0.24 (↓19%) | 2.11 ± 0.51 | 0.86 ± 0.05 | 29.2 ± 3.7 | 0.04 ± 0.02 | 3.11 ± 0.62 (↓10%) |
| 200 | 0.73 ± 0.29 (↓23%) | 2.08 ± 0.51 | 0.86 ± 0.04 | 27.1 ± 2.8 | 0.04 ± 0.01 | 3.02 ± 0.72 (↓13%) |
| 1200 | 0.59 ± 0.19** (↓38%) | 1.88 ± 0.52 (↓17%) | 0.86 ± 0.05 | 31.2 ± 5.7 | 0.03 ± 0.02 | 2.64 ± 0.62** (↓24%) |
| **Dose (ppm)** | **Neu (G/L)** | **Lymph (G/L)** | **Eos (G/L)** | **Mono (G/L)** | **LUC (G/L)** | **WBC (G/L)** |
| **Week 78 (allocation A animals)** | | | | | | |
| 0 | 1.16 ± 0.42 | 2.61 ± 0.68 | 0.08 ± 0.04 | 0.14 ± 0.04 | 0.05 ± 0.02 | 4.06 ± 0.83 |
| 30 | 1.08 ± 0.48 | 2.38 ± 0.45 | 0.08 ± 0.02 | 0.12 ± 0.03 | 0.04 ± 0.02 | 3.73 ± 0.68 |
| 200 | 1.15 ± 0.41 | 2.12 ± 0.50** (↓19%) | 0.08 ± 0.03 | 0.12 ± 0.03 | 0.04 ± 0.02 | 3.53 ± 0.69** |
| 1200 | 1.29 ± 0.55 | 2.12 ± 0.57** (↓19%) | 0.08 ± 0.03 | 0.13 ± 0.05 | 0.04 ± 0.02 | 3.68 ± 0.92 |
| **Week 104 (allocation A animals)** | | | | | | |
| 0 | 1.19 ± 0.52 | 2.31 ± 0.70 | 0.09 ± 0.05 | 0.13 ± 0.05 | 0.03 ± 0.01 | 3.76 ± 1.04 |
| 30 | 1.36 ± 1.28 | 2.22 ± 0.50 | 0.09 ± 0.06 | 0.15 ± 0.09 | 0.03 ± 0.01 | 3.86 ± 1.66 |
| 200 | 1.38 ± 0.59 | 2.22 ± 0.66 | 0.09 ± 0.04 | 0.15 ± 0.06 | 0.03 ± 0.01 | 3.87 ± 1.13# |
| 1200 | 1.82 ± 1.15* (↑53%) | 2.18 ± 0.67 | 0.10 ± 0.04 | 0.16 ± 0.07 | 0.03 ± 0.01 | 4.30 ± 1.71 |

G: giga ($10^9$); a: Clotting assay, thromboplastin from rabbit brain tissue, results as ratio of normal activity; *: significant at 5%; **: significant at 1%; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 354-368 of the study report. No standard deviation values were provided in the study report and thus were calculated by the reviewer. Weeks 13, 26 and 52, n = 20. Weeks 78 and 104, n = 50.

**2. Blood chemistry findings**

The following changes after 52 weeks were considered to be related to treatment with fluensulfone:

- Statistically significantly increased cholesterol concentration (53%) in males at 1200 ppm;

- Statistically significantly increased triglyceride concentration in males (59%) and females (33%) at 1200 ppm;

- Statistically significantly increased phospholipid concentration in males (44%) and females (26%) at 1200 ppm;

- Statistically significantly increased Aspartate aminotransferase (ASAT) concentration in males at 1200 ppm (29%);

- Statistically significantly decreased Aspartate aminotransferase (ASAT) concentration in females at 1200 ppm (23%);

- Decreased Alanine aminotransferase (ALAT) concentration in females at 200 and 1200 ppm (19 and 29%, respectively), statistically significant at 1200 ppm;

- Statistically significantly increased Lactate dehydrogenase (LDH) concentration in males at 1200 ppm (65%);

- Statistically significantly increased Alkaline phosphatase (ALP) in males at 1200 ppm (22%);

- Statistically significantly increased Creatine kinase (CK) in males at 1200 ppm (61%);

- A dose-related and statistically significant increase of the sodium (Na) concentration in all treated male groups (1-4 %), and females at 200 and 1200 ppm (2-5%);

- Increased potassium (K) concentration in males at 1200 ppm (12%);

- Statistically significantly increased chloride (Cl) concentration in females at 200 and 1200 ppm (2-4%) and in all male dose groups (1-3%);

- Statistically significantly increased calcium (Ca) concentration in males at 200 and 1200 ppm (2 and 7% respectively) and all treated female groups (3-5%);

- Statistically significantly increased phosphorus (P) concentration in males at 200 and 1200 ppm (10 and 11%, respectively);

- A dose-related increase of the total protein concentration in all treated male groups (3-12%), attaining statistical significance at 1200 ppm;

- Statistically significantly increased albumin concentration in males at 1200 ppm (8%);

- Statistically significantly increased globulin concentration in all male dose groups (6-18%).

In most of the parameters similar changes were also recorded after 13 and 26 weeks.

Changes in several other parameters achieving statistical significance were considered unrelated to treatment since they lacked a dose-relationship and were clearly within the historical control range.

## Table 11: Selected clinical chemistry parameters – Male rats (n = 20)

| Parameter | Study Week | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 30 | 200 | 1200 |
| Cholesterol (mmol/L) | 13 | 2.15 ± 0.25 | 2.06 ± 0.31 | 2.29 ± 0.48 | 2.86 ± 0.51** (↑33%) |
| | 26 | 2.47 ± 0.35 | 2.49 ± 0.38 | 2.60 ± 0.55 | 3.50 ± 0.58** (↑42%) |
| | 52 | 2.50 ± 0.38 | 2.76 ± 0.56 | 2.82 ± 0.68 | 3.83 ± 0.77** (↑53%) |
| Triglycerides (mmol/L) | 13 | 0.33 ± 0.09 | 0.33 ± 0.11 | 0.36 ± 0.18 | 0.47 ± 0.28* (↑42%) |
| | 26 | 0.39 ± 0.10 | 0.43 ± 0.13 | 0.46 ± 0.18 | 0.66 ± 0.34** (↑69%) |
| | 52 | 0.66 ± 0.22 | 0.82 ± 0.33 | 0.72 ± 0.33 | 1.05 ± 0.50** (↑59%) |
| Phospholipids (mmol/L) | 13 | 2.06 ± 0.22 | 2.06 ± 0.26 | 2.18 ± 0.32 | 2.70 ± 0.40** (↑31%) |
| | 26 | 1.83 ± 0.22 | 1.88 ± 0.24 | 1.93 ± 0.30 | 2.57 ± 0.39** (↑40%) |
| | 52 | 1.83 ± 0.24 | 2.05 ± 0.35 | 2.05 ± 0.32 | 2.63 ± 0.50** (↑44%) |
| ASAT (U/L) | 13 | 82.1 ± 11.7 | 82.4 ± 15.9 | 79.8 ± 18.9 | 87.0 ± 14.4 |
| | 26 | 78.8 ± 11.9 | 80.1 ± 10.3 | 89.5 ± 31.8 | 89.9 ± 14.9 (↑14%) |
| | 52 | 70.9 ± 10.6 | 77.6 ± 12.0 | 79.7 ± 16.4 | 91.8 ± 17.8** (↑29%) |
| ALAT (U/L) | 13 | 36.8 ± 7.4 | 36.3 ± 8.9 | 38.1 ± 8.5 | 34.8 ± 7.1 |
| | 26 | 32.8 ± 6.3 | 33.9 ± 4.2 | 34.8 ± 6.1 | 36.0 ± 5.7 |
| | 52 | 30.7 ± 5.9 | 34.5 ± 5.0 | 31.5 ± 3.6 | 31.8 ± 8.0 |
| LDH (U/L) | 13 | 173.9 ± 82.6 | 107.0 ± 21.5** | 114.8 ± 19.5** | 165.6 ± 45.7 |
| | 26 | 166.2 ± 51.9 | 162.6 ± 42.8 | 192.6 ± 166.3 | 269.4 ± 76.1** (↑62%) |
| | 52 | 109.7 ± 21.8 | 126.8 ± 28.4 | 123.3 ± 27.7 | 181.3 ± 40.8** (↑65%) |
| ALP (U/L) | 13 | 64.6 ± 18.3 | 59.6 ± 9.7 | 59.4 ± 6.5 | 65.3 ± 13.1 |
| | 26 | 46.4 ± 12.9 | 45.6 ± 7.0 | 46.6 ± 5.7 | 52.2 ± 9.7 (↑13%) |
| | 52 | 46.7 ± 11.1 | 48.6 ± 7.6 | 49.3 ± 9.3 | 57.0 ± 13.2** (↑22%) |
| CK (U/L) | 13 | 171.9 ± 60.3 | 138.1 ± 30.2 | 128.5 ± 39.5 | 195.4 ± 120.4 |
| | 26 | 165.3 ± 92.1 | 132.1 ± 45.2 | 154.0 ± 78.8 | 168.6 ± 51.3 |
| | 52 | 91.4 ± 27.4 | 97.0 ± 25.2 | 97.8 ± 26.4 | 146.7 ± 64.6** (↑61%) |
| Na (mmol/L) | 13 | 151.8 ± 7.7 | 148.9 ± 4.1 | 148.5 ± 2.9 | 149.9 ± 5.4 |
| | 26 | 145.5 ± 0.9 | 145.6 ± 0.8 | 147.0 ± 1.4** (↑1%) | 149.2 ± 1.0** (↑3%) |
| | 52 | 146.2 ± 1.2 | 147.2 ±0.9* (↑1%) | 149.2 ± 1.5** (↑2%) | 152.0 ± 1.7** (↑4%) |
| K (mmol/L) | 13 | 4.01 ± 0.41 | 3.91 ± 0.20 | 3.90 ± 0.19 | 4.05 ± 0.31 |
| | 26 | 3.81 ± 0.23 | 3.79 ± 0.21 | 3.88 ± 0.29 | 4.11 ± 0.28** (↑8%) |
| | 52 | 3.83 ± 0.19 | 3.88 ± 0.20 | 3.93 ± 0.23 | 4.27 ± 0.34** (↑12%) |
| Cl (mmol/L) | 13 | 108.4 ± 5.6 | 107.2 ± 3.1 | 106.6 ± 2.1 | 105.5 ± 3.7* (↓3%) |
| | 26 | 102.7 ± 0.9 | 102.9 ± 0.9 | 103.5 ± 1.8 | 103.0 ± 1.2 |
| | 52 | 102.6 ± | 104 ± 1.0** (↑1%) | 106.1 ± 1.6** (↑3%) | 105.3 ± 1.3** (↑3%) |
| Ca (mmol/L) | 13 | 2.77 ± 0.07 | 2.76 ± 0.08 | 2.74 ± 0.07 | 2.83 ± 0.06 |
| | 26 | 2.75 ± 0.08 | 2.76 ± 0.05 | 2.74 ± 0.09 | 2.89 ± 0.06** (↑5%) |
| | 52 | 2.74 ± 0.06 | 2.77 ± 0.06 | 2.80 ± 0.05** (↑2%) | 2.94 ± 0.07** (↑7%) |
| P (mmol/L) | 13 | 2.01 ± 0.17 | 2.01 ± 0.16 | 2.03 ± 0.20 | 2.18 ±** (↑8%) |
| | 26 | 1.76 ± 0.15 | 1.82 ± 0.16 | 1.74 ± 0.19 | 1.98 ± 0.15** (↑12%) |
| | 52 | 1.41 ± 0.17 | 1.47 ± 0.15 | 1.55 ± 0.14* (↑10%) | 1.57 ± 0.16** (↑11%) |
| Protein (g/L) | 13 | 74.44 ± 5.90 | 72.43 ± 4.07 | 71.34 ± 2.71 | 75.45 ± 4.82 |
| | 26 | 73.87 ± 2.28 | 74.27 ± 2.79 | 74.57 ± 2.65 | 80.53 ± 3.16** |
| | 52 | 73.96 ± 1.80 | 75.88 ± 2.32* (↑3%) | 76.64 ± 2.23** (↑4%) | 82.91 ± 2.68** (↑12%) |

| Parameter | Study Week | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 30 | 200 | 1200 |
| Albumin (g/L) | 13 | 45.45 ± 2.79 | 44.27 ± 1.60 | 43.88 ± 1.33* | 46.30 ± 1.97 |
| | 26 | 42.84 ± 1.35 | 42.81 ± 1.53 | 43.37 ± 1.52 | 46.02 ± 1.20** (↑7%) |
| | 52 | 42.59 ± 1.41 | 42.40 ± 1.43 | 43.48 ± 1.53 | 46.00 ± 1.50** (↑8%) |
| Globulin (g/L) | 13 | 28.99 ± 3.61 | 28.17 ± 3.13 | 27.46 ± 1.98 | 29.04 ± 3.30 |
| | 26 | 31.03 ± 1.78 | 31.46 ± 2.26 | 31.20 ± 2.04 | 34.52 ± 2.73** (↑11%) |
| | 52 | 31.37 ± 1.83 | 33.48 ± 2.06** (↑7%) | 33.16 ± 2.09* (↑6%) | 36.91 ± 2.52** (↑18%) |

m: milli ($10^{-3}$); mol: mole; U: unit; g: gram; *: significant at 5%; **: significant at 1%; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 372-377 of the study report. No standard deviation values were provided in the study report and were calculated by the reviewer.

## Table 12: Selected clinical chemistry parameters – Female rats (n = 20)

| Parameter | Study Week | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 30 | 200 | 1200 |
| Cholesterol (mmol/L) | 13 | 1.88 ± 0.34 | 1.64 ± 0.36 | 2.02 ± 0.47 | 2.16 ± 0.44 |
| | 26 | 2.40 ± 0.45 | 2.02 ± 0.44 | 2.58 ± 0.69 | 2.53 ± 0.50 |
| | 52 | 2.25 ± 0.53 | 2.11 ± 0.63 | 2.63 ± 0.60 | 2.67 ± 0.59 |
| Triglycerides (mmol/L) | 13 | 0.24 ± 0.05 | 0.24 ± 0.06 | 0.27 ± 0.07 | 0.26 ± 0.06 |
| | 26 | 0.32 ± 0.07 | 0.33 ± 0.07 | 0.35 ± 0.10 | 0.32 ± 0.06 |
| | 52 | 0.30 ± 0.08 | 0.33 ± 0.10 | 0.35 ± 0.08 | 0.40 ± 0.14** (↑33%) |
| Phospholipids (mmol/L) | 13 | 2.19 ± 0.35 | 2.09 ± 0.36 | 2.32 ± 0.45 | 2.51 ± 0.41* (↑15%) |
| | 26 | 2.29 ± 0.34 | 2.00 ± 0.37 | 2.41 ± 0.52 | 2.35 ± 0.41 |
| | 52 | 1.96 ± 0.37 | 1.97 ± 0.49 | 2.21 ± 0.60 | 2.47 ± 0.39** (↑26%) |
| ASAT (U/L) | 13 | 90.6 ± 19.0 | 79.0 ± 12.6* | 84.3 ± 15.3 | 79.6 ± 11.5 (↓12%) |
| | 26 | 94.0 ± 13.9 | 90.9 ± 38.7 | 85.6 ± 14.2 | 75.7 ± 6.0* (↓19%) |
| | 52 | 116.2 ± 39.4 | 101.5 ± 25.6 | 105.3 ± 28.4 | 89.3 ± 25.7* (↓23%) |
| ALAT (U/L) | 13 | 34.4 ± 9.0 | 29.2 ± 10.8 | 27.4 ± 3.4* (↓20%) | 28.1 ± 4.0* (↓18%) |
| | 26 | 30.7 ± 7.7 | 27.6 ± 8.1 | 28.1 ± 4.5 (↓8%) | 25.3 ± 3.7* (↓18%) |
| | 52 | 40.4 ± 11.9 | 38.5 ± 12.0 | 32.9 ± 9.6 (↓19%) | 28.6 ± 6.0** (↓29%) |
| LDH (U/L) | 13 | 125.0 ± 24.0 | 115.9 ± 23.2 | 121.0 ± 24.1 | 157.8 ± 55.5* (↑26%) |
| | 26 | 193.6 ± 89.4 | 189.9 ± 139.1 | 143.4 ± 38.7 | 179.6 ± 80.5 |
| | 52 | 131.3 ± 52.5 | 126.6 ± 39.5 | 160.6 ± 110.7 | 149.9 ± 83.1 |
| ALP (U/L) | 13 | 31.3 ± 8.2 | 27.9 ± 6.4 | 28.0 ± 7.3 | 34.7 ± 9.4 |
| | 26 | 19.7 ± 4.9 | 17.4 ± 4.1 | 17.0 ± 4.2 | 19.4 ± 4.9 |
| | 52 | 17.8 ± 5.6 | 17.0 ± 3.6 | 18.1 ± 4.2 | 18.5 ± 4.3 |
| CK (U/L) | 13 | 132.7 ± 39.7 | 140.0 ± 68.1 | 138.9 ± 57.2 | 153.3 ± 60.8 (↑15%) |
| | 26 | 136.0 ± 49.9 | 116.1 ± 48.4 | 120.4 ± 39.9 | 135.8 ± 57.8 |
| | 52 | 108.5 ± 94.9 | 97.6 ± 55.9 | 123.8 ± 68.7 | 124.6 ± 81.3 |
| Na (mmol/L) | 13 | 148.8 ± 2.5 | 144.9 ± 2.0** | 146.7 ± 1.4** | 148.9 ± 1.1 |
| | 26 | 148.1 ± 1.8 | 143.1 ± 1.1** | 144.9 ± 1.5** | 146.4 ± 1.1** |
| | 52 | 144.7 ± 1.4 | 145.0 ± 0.8 | 147.2 ± 1.7** (↑2%) | 151.5 ± 1.3** (↑5%) |
| K (mmol/L) | 13 | 3.48 ± 0.32 | 3.26 ± 0.28 | 3.34 ± 0.30 | 3.52 ± 0.31 |
| | 26 | 3.19 ± 0.29 | 3.29 ± 0.40 | 3.29 ± 0.27 | 3.43 ± 0.24 |
| | 52 | 3.44 ± 0.32 | 3.48 ± 0.31 | 3.53 ± 0.25 | 3.51 ± 0.38 |

| Parameter | Study Week | Dose (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 30 | 200 | 1200 |
| Cl (mmol/L) | 13 | 108.0 ± 2.2 | 105.5 ± 1.7** | 106.2 ± 1.8** | 107.1 ± 1.4 |
| | 26 | 104.8 ± 1.7 | 102.3 ± 1.5** | 103.2 ± 1.6** | 103.9 ± 1.3 |
| | 52 | 102.6 ± 1.8 | 102.8 ± 1.1 | 104.8 ± 2.1** (↑2%) | 106.5 ± 1.4** (↑4%) |
| Ca (mmol/L) | 13 | 2.77 ± 0.09 | 2.66 ± 0.11** | 2.68 ± 0.07** | 2.70 ± 0.09 |
| | 26 | 2.82 ± 0.10 | 2.76 ± 0.08 | 2.78 ± 0.06 | 2.79 ± 0.06 |
| | 52 | 2.73 ± 0.07 | 2.80 ± 0.10* (↑3%) | 2.80 ± 0.08* (↑3%) | 2.87 ± 0.09** (↑5%) |
| P (mmol/L) | 13 | 1.41 ± 0.22 | 1.46 ± 0.23 | 1.61 ± 0.32* | 1.57 ± 0.21 |
| | 26 | 1.47 ± 0.36 | 1.45 ± 0.28 | 1.44 ± 0.22 | 1.36 ± 0.26 |
| | 52 | 1.32 ± 0.29 | 1.35 ± 0.18 | 1.40 ± 0.14 | 1.40 ± 0.28 |
| Protein (g/L) | 13 | 77.51 ± 3.62 | 77.28 ± 3.96 | 76.55 ± 3.93 | 77.51 ± 3.16 |
| | 26 | 81.37 ± 3.92 | 78.29 ± 3.44* | 78.39 ± 4.16* | 78.46 ± 3.52* |
| | 52 | 78.12 ± 3.08 | 79.17 ± 3.96 | 79.38 ± 3.40 | 80.39 ± 3.23 |
| Albumin (g/L) | 13 | 51.50 ± 3.26 | 52.18 ± 2.35 | 50.05 ± 3..07 | 49.95 ± 2.96 |
| | 26 | 52.31 ± 3.20 | 51.52 ± 2.18 | 50.64 ± 2.97 | 49.87 ± 2.82* |
| | 52 | 48.89 ± 2.82 | 50.49 ± 2.36 | 49.51 ± 2.50 | 50.85 ± 2.44 |
| Globulin (g/L) | 13 | 25.95 ± 1.50 | 24.98 ± 1.92 | 26.50 ± 2.29 | 27.56 ± 1.09* |
| | 26 | 29.06 ± 1.75 | 26.77 ± 1.89** | 27.75 ± 2.24 | 28.59 ± 1.47 |
| | 52 | 29.23 ± 2.10 | 28.68 ± 2.40 | 30.26 ± 2.16 | 29.54 ± 2.36 |

m: milli ($10^{-3}$); mol: mole; U: unit; g: gram; *: significant at 5%; **: significant at 1%; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 372-377 of the study report. No standard deviation values were provided in the study report and were calculated by the reviewer.

## G. URINALYSIS

The levels of urinary ketones in males at 1200 ppm were statistically significantly increased at 13 and 26 weeks; the increase at 52 weeks was not statistically significant (Table 13). Ketone levels were also increased in high dose females at weeks 26 and 52, although these changes were not statistically significant. Levels of leukocytes were increased in all male dose groups at week 13, 26 and 52. Changes at the low and mid doses were not considered to be treatment-related due to low control values (compared to historical controls), lack of dose response and/or high standard deviations. No treatment-related changes in leukocytes were noted in females. Levels of leukocytes appeared to be increased at week 52 in 1200 ppm females; however, the standard deviation was notably high due to one outlier. Without the outlier value, the mean leukocyte count would be 6.6 (± 11.3).

**Table 13: Urinalysis – mean ± standard deviation of selected parameters (n = 20)**

| Males | | | Females | | |
|---|---|---|---|---|---|
| **Week 13** | **Ketones** mmol/l | **Leukocytes** per µl | **Week 13** | **Ketones** mmol/l | **Leukocytes** per µl |
| Historical Control: age 13-18 wks (n = 869) | 0.5 ± 0.6 | 24 ± 27 | Historical Control: age 13-18 wks (n = 915) | 0.2 ± 0.3 | 4 ± 10 |
| Group 1 | 0.3 ± 0.38 | 13 ± 12.8 | Group 1 | 0.1 ± 0.15 | 3 ± 7.7 |
| Group 2 | 0.2 ± 0.26 | 31 ± 37.1 (↑138%) | Group 2 | 0.1 ± 0.18 | 5 ± 10.3 |
| Group 3 | 0.6 ± 0.59 | 36* ± 33.9 (↑177%) | Group 3 | 0.1 ± 0.22 | 5 ± 10.3 |
| Group 4 | 1.0** ± 1.1 (↑233%) | 49** ± 39.3 (↑277%) | Group 4 | 0.1 ± 0.18 | 8 ± 11.8 |
| **Week 26** | | | **Week 26** | | |
| Historical Control: age 19-40 wks (n = 1600) | 0.6 ± 0.6 | 28 ± 41 | Historical Control: age 19-40 wks (n = 1630) | 0.2 ± 0.3 | 6 ± 21 |
| Group 1 | 0.6 ± 0.65 | 21 ± 21.9 | Group 1 | 0.3 ± 0.37 | 11± 12.8 |
| Group 2 | 0.8 ± 0.62 | 66 ± 109.5 (↑214%) | Group 2 | 0.3 ± 0.25 | 11 ± 23.6 |
| Group 3 | 1.1 ±0.65 | 45 ± 37.7 (↑114%) | Group 3 | 0.3 ± 0.38 | 18 ± 30.5 |
| Group 4 | 2.1** ± 1.8 (↑250%) | 63* ± 43.3 (↑200%) | Group 4 | 0.4 ± 0.52 (↑33%) | 5 ± 10.3 |
| **Week 52** | | | **Week 52** | | |
| Historical Control: age 41-70 wks (n = 259) | 0.5 ± 0.4 | 102 ± 156 | Historical Control: age 41-70 wks (n = 261) | 0.3 ± 0.3 | 58 ± 121 |
| Group 1 | 1.4 ± 1.3 | 78 ± 106.7 | Group 1 | 0.3 ± 0.39 | 14 ± 24.6 |
| Group 2 | 0.8 ± 0.63 | 124 ± 170.9 (↑59%) | Group 2 | 0.3 ± 0.38 | 9 ± 12.2 |
| Group 3 | 1.0 ± 0.55 | 114 ± 169.8 (↑46%) | Group 3 | 0.3 ± 0.47 | 18 ± 30.5 |
| Group 4 | 2.3 ± 1.9 (↑64%) | 146 ± 156.1 (↑87%) | Group 4 | 0.4 ± 0.36 (↑33%) | 31 ± 110.9 |

Data obtained from pages 383 – 386 of the study report. ( ) = % difference from control, calculated by the reviewer. *: significant at 5%; **: significant at 1%; standard deviations calculated by PMRA reviewer.

## H. SACRIFICE AND PATHOLOGY

### 1. Organ Weights

After 52 weeks, the absolute and relative weights of livers, kidneys and adrenals were increased in males at 1200 ppm (Table 14). The kidney to body weight ratio was increased (9%) and the kidney to brain weight ratio was slightly increased (4%) in females at 1200 ppm (Table 15). The increased heart to body weight ratio in males at 1200 ppm was considered to be due to the changes in body weights.

After 104 weeks, the absolute and relative weights of livers were increased in females at 1200 ppm (Table 17). Absolute and relative kidney weights in females at 1200 ppm were also increased (only relative to body weight was statistically significant). Decreases in absolute and relative spleen and adrenal weights were recorded at all dose levels in male groups but were not considered to be treatment related due to lack of dose response (Table 16). In males at 1200 ppm (body weight-) relative brain, heart, liver and kidney weights were increased. These increases were considered to be due to lower body weights recorded in this group.

**Table 14: Selected organ weights at week 52 – Male rats (Mean ± SD)**

| Organ | 0 ppm n = 20 | 30 ppm n = 20 | 200 ppm n = 20 | 1200 ppm n = 20 |
|---|---|---|---|---|
| Body weight | 595.8 ± 63.9 | 595.0 ± 65.1 | 592.4 ± 68.6 | 560.8 ± 50.0 (↓6%) |
| Brain Abs. | 2.20 ± 0.09 | 2.23 ± 0.09 | 2.19 ± 0.10 | 2.19 ± 0.07 |
| Rel. to body wt (%) | 0.37 ± 0.04 | 0.38 ± 0.04 | 0.37 ± 0.04 | 0.39 ± 0.03 |
| Heart Abs. | 1.25 ± 0.15 | 1.31 ± 0.16 | 1.28 ± 0.15 | 1.32 ± 0.16 |
| Rel. to body wt (%) | 0.21 ± 0.02 | 0.22 ± 0.02 | 0.22 ± 0.02 | 0.24 ± 0.02** (↑14%) |
| Rel. to brain wt (%) | 56.67 ± 5.73 | 58.78 ± 5.85 | 58.19 ± 5.81 | 60.38 ± 6.86 |
| Liver Abs. | 14.34 ± 1.51 | 15.55 ± 2.24 | 14.60 ± 2.39 | 16.03 ± 2.05* (↑12%) |
| Rel. to body wt (%) | 2.42 ± 0.30 | 2.61 ± 0.20 | 2.46 ± 0.23 | 2.86 ± 0.30** (↑18%) |
| Rel. to brain wt (%) | 651.91 ± 65.49 | 698.72 ± 100.33 | 664.62 ± 90.93 | 732.47 ± 88.63** (↑12%) |
| Kidneys Abs. | 2.48 ± 0.23 | 2.61 ± 0.30 | 2.56 ± 0.34 | 2.95 ± 0.36** (↑19%) |
| Rel. to body wt (%) | 0.42 ± 0.06 | 0.44 ± 0.03 | 0.43 ± 0.04 | 0.53 ± 0.05** (↑26%) |
| Rel. to brain wt (%) | 112.67 ± 9.87 | 117.31 ± 12.28 | 116.51 ± 12.66 | 134.91 ± 15.13** (↑20%) |
| Adrenals Abs. | 0.056 ± 0.007 | 0.058 ± 0.010 | 0.057 ± 0.009 | 0.064 ± 0.010* (↑14%) |
| Rel. to body wt (%) | 0.010 ± 0.002 | 0.010 ± 0.002 | 0.010 ± 0.002 | 0.011 ± 0.002** (↑10%) |
| Rel. to brain wt (%) | 2.554 ± 0.295 | 2.595 ± 0.428 | 2.610 ± 0.420 | 2.908 ± 0.458* (↑14%) |
| Spleen Abs. | 1.11 ± 0.17 | 1.07 ± 0.26 | 1.06 ± 0.19 | 1.06 ± 0.16 |
| Rel. to body wt (%) | 0.19 ± 0.03 | 0.18 ± 0.03 | 0.18 ± 0.03 | 0.19 ± 0.02 |
| Rel. to brain wt (%) | 50.34 ± 7.81 | 47.97 ± 11.01 | 48.15 ± 8.15 | 48.43 ± 7.37 |

* and **: Dunnett-test based on pooled variance significance at 5% and 1%, respectively; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 388 to 399 of the study report.

**Table 15: Selected organ weights at week 52 – Female rats (Mean ± SD)**

| Organ | 0 ppm n = 18 | 30 ppm n = 20 | 200 ppm n = 20 | 1200 ppm n = 20 |
|---|---|---|---|---|
| Body weight | 310.4 ± 33.4 | 319.9 ± 45.0 | 306.9 ± 48.5 | 287.3 ± 32.7 (↓7%) |
| Brain Abs. | 2.05 ± 0.08 | 2.02 ± 0.09 | 2.02 ± 0.08 | 1.98 ± 0.07* |
| Rel. to body wt (%) | 0.67 ± 0.07 | 0.64 ± 0.08 | 0.67 ± 0.09 | 0.70 ± 0.07 |
| Heart Abs. | 0.88 ± 0.07 | 0.84 ± 0.07 | 0.85 ± 0.09 | 0.85 ± 0.06 |
| Rel. to body wt (%) | 0.28 ± 0.02 | 0.26 ± 0.03* | 0.28 ± 0.02 | 0.30 ± 0.02 |
| Rel. to brain wt (%) | 42.75 ± 3.02 | 41.41 ± 2.81 | 42.02 ± 3.93 | 42.82 ± 2.94 |
| Liver Abs. | 8.95 ± 0.80 | 8.68 ± 0.93 | 8.79 ± 1.35 | 8.70 ± 0.95 |
| Rel. to body wt (%) | 2.89 ± 0.18 | 2.73 ± 0.22 | 2.88 ± 0.27 | 3.04 ± 0.28 |
| Rel. to brain wt (%) | 436.03 ± 37.14 | 429.86 ± 44.72 | 435.61 ± 65.14 | 438.52 ± 43.67 |
| Kidneys Abs. | 1.71 ± 0.18 | 1.64 ± 0.14 | 1.65 ± 0.22 | 1.72 ± 0.15 |
| Rel. to body wt (%) | 0.55 ± 0.05 | 0.52 ± 0.07 | 0.54 ± 0.06 | 0.60 ± 0.07* (↑9%) |
| Rel. to brain wt (%) | 83.41 ± 8.64 | 81.20 ± 5.80 | 81.96 ± 10.44 | 86.69 ± 8.56 (↑4%) |
| Adrenals Abs. | 0.073 ± 0.011 | 0.064 ± 0.012* | 0.065 ± 0.011 | 0.066 ± 0.009 |
| Rel. to body wt (%) | 0.024 ± 0.004 | 0.020 ± 0.004* | 0.021 ± 0.005 | 0.023 ± 0.003 |
| Rel. to brain wt (%) | 3.551 ± 0.514 | 3.146 ± 0.574 | 3.206 ± 0.541 | 3.327 ± 0.462 |
| Spleen Abs. | 0.79 ± 0.16 | 0.77 ± 0.12 | 0.77 ± 0.15 | 0.80 ± 0.62 |
| Rel. to body wt (%) | 0.26 ± 0.06 | 0.25 ± 0.05 | 0.25 ± 0.04 | 0.27 ± 0.18 |
| Rel. to brain wt (%) | 38.57 ± 7.12 | 38.12 ± 5.25 | 38.19 ± 7.45 | 40.43 ± 30.78 |

* and **: Dunnett-test based on pooled variance significance at 5% and 1%, respectively; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 388 to 399 of the study report.

**Table 16: Selected organ weights at week 104 – Male rats (Mean ± SD)**

| Organ | 0 ppm n = 39 | 30 ppm n = 42 | 200 ppm n = 35 | 1200 ppm n = 38 |
|---|---|---|---|---|
| Body weight | 688.8 ± 82.2 | 674.7 ± 86.1 | 651.3 ± 90.7 | 623.4 ± 96.8** (↓9%) |
| Brain Abs. | 2.26 ± 0.09 | 2.24 ± 0.11 | 2.24 ± 0.12 | 2.24 ± 0.10 |
| Rel. to body wt (%) | 0.33 ± 0.04 | 0.34 ± 0.05 | 0.35 ± 0.05 | 0.37 ± 0.06** (↑12%) |
| Heart Abs. | 1.42 ± 0.21 | 1.35 ± 0.15 | 1.36 ± 0.17 | 1.42 ± 0.16 |
| Rel. to body wt (%) | 0.21 ± 0.03 | 0.20 ± 0.04 | 0.21 ± 0.02 | 0.23 ± 0.04** (↑10%) |
| Rel. to brain wt (%) | 62.67 ± 8.57 | 60.45 ± 7.27 | 60.87 ± 6.94 | 63.16 ± 7.29 |
| Liver Abs. | 18.12 ± 3.40 | 17.12 ± 2.32 | 16.56 ± 2.88* | 18.40 ± 2.55 |
| Rel. to body wt (%) | 2.63 ± 0.33 | 2.56 ± 0.39 | 2.55 ± 0.27 | 2.98 ± 0.38** (↑13%) |
| Rel. to brain wt (%) | 799.93 ± 136.77 | 765.04 ± 109.42 | 740.53 ± 124.31 | 821.27 ± 117.29 |
| Kidneys Abs. | 3.35 ± 1.18 | 3.25 ± 1.06 | 3.06 ± 0.54 | 3.46 ± 0.42 |
| Rel. to body wt (%) | 0.48 ± 0.13 | 0.49 ± 0.20 | 0.47 ± 0.05 | 0.56 ± 0.09* (↑17%) |
| Rel. to brain wt (%) | 147.81 ± 48.86 | 145.47 ± 49.03 | 136.90 ± 23.79 | 154.35 ± 19.20 |
| Adrenals Abs. | 0.137 ± 0.428 | 0.071 ± 0.027 (↓48%) | 0.066 ± 0.012 (↓52%) | 0.087 ± 0.118 (↓37%) |
| Rel. to body wt (%) | 0.020 ± 0.065 | 0.011 ± 0.005 (↓45%) | 0.010 ± 0.002 (↓50%) | 0.014 ± 0.017 (↓30%) |
| Rel. to brain wt (%) | 5.996 ± 18.583 | 3.189 ± 1.295 (↓47%) | 2.978 ± 0.566 (↓50%) | 3.852 ± 5.010 (↓36%) |
| Spleen Abs. | 1.72 ± 0.64 | 1.45 ± 0.41* (↓16%) | 1.36 ± 0.33** (↓21%) | 1.41 ± 0.44* (↓18%) |
| Rel. to body wt (%) | 0.25 ± 0.09 | 0.21 ± 0.06 (↓16%) | 0.21 ± 0.04* (↓16%) | 0.23 ± 0.06 (↓8%) |
| Rel. to brain wt (%) | 76.30 ± 29.45 | 64.41 ± 17.82* (↓16%) | 60.93 ± 14.48** (↓20%) | 62.97 ± 19.49* (↓17%) |

* and **: Dunnett-test based on pooled variance significance at 5% and 1%, respectively. ( ) = % different from control, calculated by the reviewer. Data obtained from pages 400 to 411 of the study report.

**Table 17: Selected organ weights at week 104 – Female rats (Mean ± SD)**

| Organ | 0 ppm n = 36 | 30 ppm n = 36 | 200 ppm n = 33 | 1200 ppm n = 38 |
|---|---|---|---|---|
| Body weight | 386.4 ± 58.5 | 399.8 ± 58.8 | 387.2 ± 64.6 | 368.6 ± 59.4 (↓5%) |
| Brain Abs. | 2.06 ± 0.15 | 2.06 ± 0.09 | 2.01 ± 0.09 | 2.04 ± 0.10 |
| Rel. to body wt (%) | 0.54 ± 0.09 | 0.52 ± 0.08 | 0.53 ± 0.07 | 0.57 ± 0.09 |
| Heart Abs. | 0.97 ± 0.12 | 0.96 ± 0.09 | 0.97 ± 0.10 | 0.97 ± 0.11 |
| Rel. to body wt (%) | 0.25 ± 0.03 | 0.24 ± 0.03 | 0.25 ± 0.03 | 0.27 ± 0.03 |
| Rel. to brain wt (%) | 47.14 ± 5.92 | 46.76 ± 4.06 | 48.10 ± 4.19 | 47.68 ± 5.91 |
| Liver Abs. | 10.48 ± 1.76 | 10.64 ± 1.84 | 10.57 ± 1.70 | 11.81 ± 2.43* (↑13%) |
| Rel. to body wt (%) | 2.72 ± 0.29 | 2.67 ± 0.31 | 2.75 ± 0.35 | 3.21 ± 0.42** (↑18%) |
| Rel. to brain wt (%) | 511.98 ± 89.37 | 516.53 ± 88.76 | 524.40 ± 74.42 | 579.47 ± 127.8* (↑13%) |
| Kidneys Abs. | 2.03 ± 0.25 | 2.06 ± 0.26 | 2.06 ± 0.24 | 2.15 ± 0.29 (↑6%) |
| Rel. to body wt (%) | 0.53 ± 0.07 | 0.52 ± 0.08 | 0.54 ± 0.07 | 0.59 ± 0.07** (↑11%) |
| Rel. to brain wt (%) | 98.95 ± 11.85 | 100.18 ± 12.36 | 102.45 ± 10.35 | 105.38 ± 14.98 (↑7%) |
| Adrenals Abs. | 0.069 ± 0.016 | 0.071 ± 0.014 | 0.069 ± 0.015 | 0.067 ± 0.012 |
| Rel. to body wt (%) | 0.018 ± 0.005 | 0.018 ± 0.004 | 0.018 ± 0.005 | 0.018 ± 0.004 |
| Rel. to brain wt (%) | 3.370 ± 0.774 | 3.432 ± 0.721 | 3.448 ± 0.706 | 3.268 ± 0.648 |
| Spleen Abs. | 0.91 ± 0.18 | 0.95 ± 0.26 | 0.95 ± 0.23 | 0.95 ± 0.24 |
| Rel. to body wt (%) | 0.24 ± 0.04 | 0.24 ± 0.06 | 0.25 ± 0.07 | 0.26 ± 0.04 |
| Rel. to brain wt (%) | 44.17 ± 8.53 | 46.24 ± 12.28 | 46.95 ± 10.87 | 46.37 ± 12.46 |

* and **: Dunnett-test based on pooled variance significance at 5% and 1%, respectively; ( ) = % different from control, calculated by the reviewer. Data obtained from pages 400 to 411 of the study report.

**2. Gross and Histopathology**

No gross lesions attributed to the treatment were noted at the interim kill after 52 weeks of dosing. All gross lesions were within the range of normal background alterations.

After 104 weeks (terminal sacrifice), an increased number of focus/foci in the lungs were recorded in animals at 1200 ppm (statistically significant in males) (Table 18). Slight increases in incidences of foci and cysts in livers of females at 1200 ppm, and foci in the prostate of males at 200 and 1200 ppm were also noted.

All remaining findings recorded were regularly distributed among control and treated groups, and were considered to be within the range of normal background lesions, which may be seen in rats of the used strain and age.

**Table 18: Incidence of selected macroscopic findings after 104 weeks**

| Findings | 0 ppm n = 50 | | 30 ppm n = 50 | | 200 ppm n = 50 | | 1200 ppm n = 50 | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| Lung: Focus/foci | 3 [6%] | 4 [8%] | 5 [10%] | 8 [16%] | 5 [10%] | 9 [18%] | 15** [30%] | 11 [22%] |
| Liver: Focus/foci | 7 [14%] | 0 [0%] | 9 [18%] | 2 [4%] | 6 [12%] | 3 [6%] | 10 [20%] | 4 [8%] |
| Cysts | 0 [0%] | 3 [6%] | 1 [2%] | 1 [2%] | 1 [2%] | 1 [2%] | 1 [2%] | 8 [16%] |
| Prostate: Focus/foci | 1 [2%] | | 3 [6%] | | 5 [10%] | | 5 [10%] | |

** significant p <0.001 (Fisher's Exact). Data obtained from pages 419 – 431 and 1733 of the study report. [ ] = % incidence, calculated by the reviewer.

There were no treatment-related tumors recorded. All neoplastic findings were within the range of normal background lesions which may be recorded in animals of the used strain and age.

Non-neoplastic lesions were noted in the esophagus at sacrifice after both 52 and 104 weeks, and in the lungs, kidneys and pharynx after 104 weeks only.

After 52 weeks, hyperkeratosis of the esophagus was recorded at a minimal severity grade in animals of both sexes at 1200 ppm (Table 19). Statistical significance was recognized in both males and females by the Fisher's Exact (males $p<0.01$; females $p<0.05$) and Armitage trend test ($p<0.01$) for both sexes.

After 104 weeks, an increased incidence of hyperkeratosis was recorded in both males and females at 200 and 1200 ppm. Statistical significance was recognized in both males and females (Fisher's Exact $p<0.01$) at 1200 ppm only and there was a positive trend in both sexes by the Armitage trend test ($p<0.01$). A dose-related increase in severity was not observed.

**Table 19: Incidence / mean severity grade of hyperkeratosis of the esophagus**

| | 0 ppm | | 30 ppm | | 200 ppm | | 1200 ppm | |
|---|---|---|---|---|---|---|---|---|
| **52 weeks (N=20)** | | | | | | | | |
| | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| Grade 1 | - | - | - | - | - | - | 12** | 6* |
| Total affected | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 6 |
| Mean severity | - | - | - | - | - | - | 1.0 | 1.0 |
| **104 weeks (N=48-50)** | | | | | | | | |
| | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| Grade 1 | 3 | 1 | 2 | 3 | 7 | 6 | 20 | 14 |
| Grade 2 | - | 1 | - | - | - | 2 | - | 5 |
| Grade 3 | - | - | - | - | - | - | 1 | 1 |
| Total affected | 3 | 2 | 2 | 3 | 7 | 8 | 21** | 20** |
| Mean severity | 1.0 | 1.5 | 1.0 | 1.0 | 1.0 | 1.3 | 1.1 | 1.4 |

For details regarding tests for statistical significance, see the text above. Data obtained from pages 47, 1735, and 1744-1745 of the study report.

There were no microscopic findings in the lungs after 52 weeks of treatment. After 104 weeks, there was an increase in incidence of chronic interstitial inflammation in the lungs recorded in both males and females at 1200 ppm and females at 200 ppm, which was only statistically significant in the high dose male group as compared to the control group by Fisher's Exact pairwise comparison ($p<0.01$) (Table 20). Statistical significance was recognized in both males and females by Armitage trend test ($p<0.01$). The chronic interstitial inflammation was characterized by focal/multifocal changes consisting of interstitial or intra-alveolar inflammatory cells associated with hypertrophied reactive type II pneumocytes. These lesions were associated with foamy intra-alveolar macrophages, which were slightly increased in mean severity grade when compared to the lungs of control male and female rats. The lesions were non-hyperplastic.

**Table 20: Incidence and severity grade of microscopic findings in the lungs (104 weeks)**

| Severity | 0 ppm | | 30 ppm | | 200 ppm | | 1200 ppm | |
|---|---|---|---|---|---|---|---|---|
| | **M** | **F** | **M** | **F** | **M** | **F** | **M** | **F** |
| **Chronic interstitial inflammation** | | | | | | | | |
| Grade 1 | 1 | 3 | 3 | 1 | 1 | 4 | 3 | 7 |
| Grade 2 | 1 | - | 1 | - | 1 | 3 | 10 | - |
| Grade 3 | - | - | - | - | - | - | - | 2 |
| Total affected | 2 | 3 | 4 | 1 | 2 | **7** | **13**** | **9** |
| Mean severity | 1.5 | 1.0 | 1.3 | 1.0 | 1.5 | 1.4 | 1.8 | 1.4 |
| **Alveolar macrophages** | | | | | | | | |
| Grade 1 | 29 | 30 | 21 | 23 | 10 | 26 | 17 | 24 |
| Grade 2 | 5 | 1 | 4 | 10 | 15 | 13 | 14 | 6 |
| Grade 3 | - | - | 1 | - | 1 | - | 1 | 3 |
| Total affected | 34 | 31 | 26 | 33 | 26 | 39 | 32 | 33 |
| Mean severity | 1.1 | 1.0 | 1.2 | 1.3 | 1.7 | 1.3 | 1.5 | 1.4 |

N=49-50. For details regarding tests for statistical significance, see the text above. Data obtained from pages 46-47 and 1734, 1735, and 1744-1745 of the study report.

At 104 weeks, slight increases were observed in chronic nephropathy, tubular basophilia and mononuclear infiltrates in the kidneys and mononuclear infiltrates in the pharynx of females treated at 1200 ppm as compared to controls (Table 21). These tissues were not examined in all animals from the low- and mid-dose groups.

**Table 21: Incidence of non-neoplastic lesions in the kidney and pharynx (104 weeks)**

| Findings | 0 ppm | | 30 ppm | | 200 ppm | | 1200 ppm | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| **Kidney** – | **n = 50** | **n = 50** | **n = 13** | **n = 16** | **n = 17** | **n = 16** | **n = 50** | **n = 50** |
| Chronic nephropathy | 41 (82%) | 8 (16%) | 6 (46%) | 3 (19%) | 8 (47%) | 4 (25%) | 38 (76%) | 12 (24%) |
| Tubular basophilia | 6 (12%) | 11 (22%) | 2 (15%) | 3 (19%) | 2 (12%) | 5 (31%) | 9 (18%) | 19 (38%) |
| Mononuclear foci | 4 (8%) | 14 (28%) | 4 (31%) | 6 (38%) | 3 (18%) | 5 (32%) | 5 (10%) | 21 (42%) |
| **Pharynx** – | **n = 38** | **n =48** | **--** | **n = 11** | **--** | **n = 14** | **n = 42** | **n = 46** |
| Mononuclear infiltrates | 8 (21%) | 7 (15%) | -- | 1 (9%) | -- | 2 (14%) | 4 (10%) | 14 (30%) |

Data obtained from pages 1721 – 1783. ( ) = % incidence, calculated by the reviewer.

### 3. Additional Investigations

#### 3.1 Liver Enzyme Determinations

Slight, non- significant decreases in hepatic alanine aminotransferase (ALAT) activity were observed in males at all dose levels, but were not considered to be related to treatment due to the lack of dose response. Decreases in 7-ethoxy-resorufin O-dealkylase (CYP1A1) activities were noted in females (Table 22), reaching statistical significance at 30 and 1200 ppm, but not at 200 ppm; as the response was not dose-dependent, these decreases were not considered to be related to treatment. The phase II enzymes uridine diphosphoglucuronosyl-transferase (UDPGT) and glutathione S-transferase (GST) were induced in females at 200 ppm and in both sexes at 1200 ppm, and epoxide hydrolase (mEH) was induced in all male and female dose groups. These enzymes were more significantly induced in females than males.

**Table 22: Selected liver enzyme activities (Mean ± SD; % difference from of control)**

| Parameter | 0 ppm | | 30 ppm | | 200 ppm | | 1200 ppm | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| ALAT (U/g liver) | 93.0 ± 32.2 | 47.2 ± 10.4 | 71.7 ± 20.4 (↓23%) | 38.6 ± 9.3 | 69.8 ± 8.8 (↓25%) | 46.3 ± 7.4 | 71.7 ± 18.8 (↓23%) | 42.4 ± 2.4 |
| CYP1A1 (nmol/min/ g liver) | 2.21 ± 1.30 | 3.46 ± 1.78 | 1.91 ± 1.41 (↓14%) | 0.89 ± 0.23 (↓74%) | 2.52 ± 0.51 (↑14%) | 2.22 ± 1.64 (↓36%) | 3.94 ± 3.44 (↑78%) | 1.13 ± 0.47 (↓67%)* |
| GST (nmol/min/ g liver) | 38.7 ± 6.5 | 26.6 ± 5.3 | 40.7 ± 3.7 | 30.3 ± 3.5 | 44.2 ± 2.5 | 38.0 ± 9.0 (↑43%)* | 53.3 ± 6.0 (↑38%)** | 40.7 ± 2.4 (↑54%)** |
| UDPGT (µmol/min/ g liver) | 1.44 ± 0.25 | 0.91 ± 0.23 | 1.47 ± 0.42 | 0.98 ± 0.21 | 1.62 ± 0.21 | 1.27 ± 0.65 (↑40%) | 2.36 ± 0.61 (↑64%)* | 1.67 ± 0.35 (↑84%)* |
| mEH (nmol/min/ g liver) | 177 ± 21.4 | 43.6 ± 15.8 | 242 ± 80.8 (↑37%) | 54.9 ± 16.6 (↑26%) | 215 ± 42.2 (↑22%) | 92.5 ± 29.6 (↑112%)** | 351 ± 43.6 (↑98%)** | 97.3 ± 27.3 (↑123%)** |

ALAT: alanine aminotransferase in 100g supernatants; GST: cytosolic glutathione S-transferase activity; UDPGT: microsomal uridine diphospho-glucuronosyl transferase activity; mEH: microsomal epoxide hydrolase activity; * and **: significantly different from control (Dunnett's t-test) at 5% and 1%, respectively. Data obtained from pages 3512-3539 of the study report. n = 5. () = % difference from control, calculated by the reviewer.

### 3.2 Fluoride Determination

Treatment with fluensulfone for 52 weeks resulted in statistically significant increases of fluoride content of ashes from bones and teeth of male and female rats receiving 200 or 1200 ppm fluensulfone in the diet, compared to controls (Table 23). Comparatively slight increases in fluoride content from bones were noted in males and females at 30 ppm. No increase of the fluoride content in ashes from teeth was recorded in animals treated with 30 ppm, compared to controls.

After 104 weeks of dosing, statistically significant increases of fluoride content in ashes from bones and teeth of male and female rats receiving 200 or 1200 ppm, and from bones only in rats receiving 30 ppm, compared to controls, were recorded. Comparatively slight increases in fluoride content from teeth were noted in males and females at 30 ppm.

**Table 23: Fluoride content – ppm (Mean ± Relative SD %)**

| Parameter | 0 ppm | | 30 ppm | | 200 ppm | | 1200 ppm | |
|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F |
| **52 weeks** | | | | | | | | |
| Bones | 577.1 ± 8.0 | 579.8 ± 6.7 | 647.2 ± 4.4 (↑12%) | 743.9 ± 8.6 (↑28%) | 1682 ± 9.3* (↑192%) | 1896 ± 9.9* (↑227%) | 5582 ± 8.2* (↑867%) | 5316 ± 7.1* (↑817%) |
| Teeth | 93.48 ± 13.7 | 123.5 ± 9.8 | 90.12 ± 15.2 | 99.75 ± 9.2 | 519.6 ± 9.4* (↑456%) | 640.2 ± 14.0* (↑418%) | 1854 ± 19.9* (↑1883%) | 2006 ± 13.7* (↑1524%) |
| **104 weeks** | | | | | | | | |
| Bones | 398.7 ± 15.4 | 642.4 ± 7.9 | 720.2 ± 11.5* (↑81%) | 945.0 ± 6.9* (↑47%) | 2072 ± 11.9* (↑420%) | 2560 ± 7.5* (↑299%) | 6567 ± 3.6* (↑1547%) | 6258 ± 5.0* (↑874%) |
| Teeth | 135.3 ± 34.1 | 126.0 ± 9.7 | 162.9 ± 17.5 (↑20%) | 209.2 ± 17.9 (↑66%) | 484.4 ± 13.9* (↑258%) | 567.5 ± 11.6* (↑350%) | 1740 ± 16.0* (↑1186%) | 1959 ± 8.8* (↑1455%) |

*: significantly different from control (Dunnett's t-test) at 5%. Data obtained from pages 3571-3578 of the study report. ( ) = % different from control, calculated by the reviewer.

## III. APPLICANT'S CONCLUSIONS

The NOAEL (No Observed Adverse Effect Level) for chronic toxicity was established at 200 ppm (11 mg/kg bw/day in males and 13.1 mg/kg bw/day in females) based on hematological findings, hyperkeratosis of the esophagus and decreased body weights and body weight gains at 1200 ppm. The effects on electrolytes, in some cases affecting all dose levels in a single gender (sodium in males, calcium in females) and the increase in protein concentration in males at all dose levels were not considered to be adverse due to the absence of other correlating findings.

The NOAEL for chronic treatment was established at 30 ppm (1.4 mg/kg bw/day in males and 1.7 mg/kg bw/day in females) based on chronic interstitial inflammation in the lungs of females, decreased body weight and body weight gain in males, and decreased hemoglobin concentration and hemoglobin concentration distribution width in females at 200 ppm.

Fluensulfone showed no carcinogenic potential in rats.

## IV. EVALUATION, SUMMARY AND CONCLUSIONS

A. **Name of authority:** Primary review: Health Evaluation Directorate, Pest Management Regulatory Agency (PMRA), Health Canada

Secondary review: Health Effects Division, Office of Pesticides Program (OPP), United States Environmental Protection Agency and Office of Chemical Safety (OCS), Australian Pesticides and Veterinary Medicines Authority

B. **Reviewer's Comments**: This study in is classified as acceptable/guideline (fully reliable) and satisfies the guideline requirement for a combined chronic/carcinogenicity study (OPPTS 870.4300; OECD 453) in rats.

C. **Conclusions**: For the current study, the Applicant established separate NOAELs for chronic toxicity and chronic treatment; however, this is not common practice for the regulatory authorities. In this chronic/oncogenicity study, the reviewer is in agreement with establishing the NOAEL at 200 ppm (9.6 mg/kg bw/day in males and 11.6 mg/kg bw/day in females) based on a LOAEL of 1200 ppm (57.7/69.9 mg/kg/day, M/F).. The increased incidence and severity of interstitial inflammation in the lungs of female rats at 200 and 1200 ppm were not considered treatment related. Interstitial inflammatory cells are a common observation in older rats, and ancillary pathologic changes associated with cell injury (e.g. pneumocyte hyperplasia or necrosis) were not observed here. However, there was a statistically significant increase in the incidence (2/50 in controls and 13/50 at 1200 ppm) and increased severity (1.5/4 in controls and 1.8/4 at 1200 ppm) of chronic interstitial inflammation at 1200 ppm in males that was considered treatment related. Additional adverse, treatment-related effects were noted in high dose animals, including hematological and clinical chemistry findings, and histopathological findings in the esophagus. In both males and females at 52 and 104 weeks, statistically significant, dose-related increases in fluoride levels compared to controls in the bone (192 to 1547%) and teeth (258 to 1883%) were observed at 200 and 1200 ppm; slight increases (12 to 88%) were seen in bone and teeth at 30 ppm. However, no structural changes related to fluoride deposition were reported in either tissue. The 11%/15% decrease in body weight/weight gain in 1200 ppm males and 6%/15% decrease in 1200 ppm females indicate that MTD (maximum tolerated dose) was reached at this this dose level. No oncogenic potential was noted in the study.

OCS does not consider that the decreases in male body weight and body weight gain at 200 ppm are of a sufficient magnitude to be considered adverse i.e. both up to 7% max over the study duration with decreases of ≤5% at study termination. OCS considers that the NOAEL in males is 200 ppm (9.6 mg/kg bw/day) based on the magnitude of the decreases seen in male body weight (11%) and body weight gain (21%), hematology and clinical chemistry findings, effects on liver, kidney and adrenal weight, and histopathological changes in the lungs and esophagus.

D. **Deficiencies**: None.